The Neurosciences from Basic Research to Therapy

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Edited by
Norbert Elsner and Herbert Zimmermann

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About the PDF version of the conference proceedings volume

Dear reader,

this file contains the abstracts of all the main lectures, talks and posters as published in the printed conference procceedings volume. It is available for download at

http://www.neuro.uni-goettingen.de/proceedings.html.

In addition, page III of this file contains an abstract for a poster contributed to the subject area "Muscle,motor and sensorimotor systems":

Analysis of saccade-related fastigial nucleus activity in the alert monkey

by Y. Guan, J.F. Kleine and U. Büttner.

This poster was accidentally unregistered. Therefore, it was neither listed in the programme booklet nor was an abstract included in the printed conference proceedings volume.

By decision of their authors, the posters listed below were not displayed among the contributions to the subject area or symposium they are assigned to in the printed conference proceedings volume and in this file:

Poster

237 B.Schönebeck,X.Zhu,H.Lübbert and C.Stichel,Bochum and Leverkusen:

Serum and glucocorticoid-regulated kinase:A differentially expressed gene in a MPTP-model of Parkinson`s disease

Was displayed among the contributions to subject area "Mechanoreception".

Posters

274 N.Arai,S.Okabe,N.Kobayashi-Iwata, T.Furubayashi, K.Machii, R.Hanajima, Y.Terao, K.Yuasa, S.Tsuji and Y.Ugawa, Tokyo (Japan):

Comparison between monophasic and biphasic transcranial magnetic stimulation of the human motor cortex

and

275 T.Furubayashi,Y.Terao,N.Arai,S.Okabe,H.Mochizuki,S.Tsuji and Y.Ugawa, Tokyo (Japan):

Effects of transient transcranial direct currents over the human hand motor area

Were displayed among the contributions to Satellite Symposium C, "2.International transcranial magnetic stimulation (TMS)and transcranial direct current stimulation (tDCS) Symposium".

Poster

451 E.Claes,M.Seeliger,M.Biel,P.Humphries and S.Haverkamp,Frankfurt, Tübingen,München and Dublin (Ireland)

Morphological alterations in the retina of CNG3 $^{-/-}$ Rho $^{-/-}$ double mutant mice

Was displayed among the contributions to subject area "Visual systems of vertebrates: Periphery"

Posters

528 N.Dambeck,K.Stock,J.Weidemann,I.G.Meister,H.Foltys and B.Boroojerdi, Aachen

Investigating phosphene elicitation with the paired-pulse paradigm

and

1013 N.Dambeck,M.Wienemann,J.Weidemann,I.G.Meister,R.Töpper and B.Boroojerdi,Aachen and Hamburg

Visuo-spatial attention in the vertical dimension: A TMS study

Were displayed among the contributions to Satellite Symposium C, "2.International transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) Symposium".

Yours faithfully

The Organisers of the 29th Göttingen Neurobiology Conference

Analysis of saccade-related fastigial nucleus activity in the alert monkey

Y. Guan, J.F. Kleine and U. Büttner

Dept. of Neurology, Ludwig-Maximilians-Universität München, München, Germany

A remarkable role in saccade control is played by the cerebellar caudal fastigial nucleus (fastigial oculomotor region, FOR), which lies under the cerebellar vermis. Its bursting activity is related to many saccade properties, such as direction, amplitude and duration. The precise mechanism by which FOR bursting influences a saccade is under debate.
We have investigated (1) whether or not the initial eye position has an effect on FOR bursting for ipsilateral and contralateral saccades, (2) what the difference is between centripetal and centrifugal saccades, and (3) if FOR neurones influence acceleration and deceleration of a saccade and, if so, in what way.
For this purpose, a 9-point horizontal start position training paradigm has been applied, using a 3x3 square grid spaced at 16° intervals. Based on observations on 75 saccade-related FOR neurones, our results permit the following conclusions.

(1) Ipsilateral saccades clearly differ from contralateral ones. Individual neurone analysis as well as population burst analysis based on averaging the data across neurones show that neither the vertical nor the horizontal component of the initial eye position substantially influences saccade-related bursting of the FOR neurone population.
(2) Centripetal and centrifugal saccades do not differ as to FOR bursting.
(3) Although most FOR neurones do not receive information about eye position, they seem to play a crucial role in acceleration and/or deceleration of saccade movement.

Finally, as the discharge patterns of individual neurones are highly variable, with prominent differences in both latency and amplitude, we propose that modification of the relative contributions of individual FOR neurones to the total FOR neurone population response may provide a mechanism for the adaptive control of saccade metrics in the monkey.

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Remarks on the cover illustration: Photomontage of a DiI-labelled neuron from human prefrontal cortex and a human brain. The post-mortem specimen was kindly provided by Dr. D. Senitz, Universität Würzburg. About 100 optical sections were scanned through the tissue with a confocal laser scanning microscope and volume rendered using a 3D image software. Copyright: Werner Zuschratter, Leibniz Institut für Neurobiologie, Magdeburg.

Remarks on the frontispiece: Adaptation has traditionally been used to study early sensory processing including the behaviour of single cells. During the last few years, however, it has become evident that adaptation also plays a major role in high-level cognitive processes and that it can be employed to study phenomena such as face recognition or biological motion perception. The first symposium of the 29th Göttingen Neurobiology Conference is trying to bridge the range between single cell physiology and high level cognitive phenomena by emphasizing the generality of adaptive mechanisms. Copyright of figure: Nikolaus Troje, Fakultät für Psychologie, Ruhr-Universität Bochum.

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Plenary Lectures and Symposia

Poster Contributions

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0254–0279	Muscle, motor and sensorimotor systems
0280–0287	Rhythmogenesis and motor pattern generation
0288–0311	Audition, vibration and communication in invertebrates
0312–0317	Audition and vocalization in lower vertebrates
0318–0329	Audition and vocalization in birds and mammals: Periphery
0330–0371	Audition and vocalization in birds and mammals: CNS and perception
0372–0387	Lateral line systems; Vestibular systems
0388–0442	Chemosensory and thermosensory systems
0443–0468	Visual systems of invertebrates: Periphery
0469–0500	Visual systems of invertebrates: Central areas and perception
0501–0526	Visual systems of vertebrates: Periphery
0527–0560	Visual systems of vertebrates: Central areas and perception
0561–0581	Visual systems of vertebrates: Development and regeneration
0582–0606	Cortex and Cerebellum
0607–0634	Hippocampus and Limbic system
0635–0676	Learning and Memory
0677–0692	Neuroanatomical studies
0693–0702	Neurohistochemical studies
0703–0710	Neurochemistry
0711–0760	Synapses and transmitters
0761–0785	Neuropeptides and neuromodulation
0786–0843	Ion channels and receptors
0844–0867	Neuropharmacology and -toxicology
0868–0881	Cell and tissue cultures
0882–0900	Glia cells; Myelin
0901–0936	Neuronal development
0937–0976	Regeneration and plasticity
0977–0983	Neurogenetics
0984–1001	Neuropathology
1002–1006	Neural-immune interactions
1007–1012	Neuroendocrinology
1013–1030	Neuropsychology and psychophysics
1031–1058	Neuronal networks theory and modeling
1059–1087	Methods and demonstrations

Lectures and Poster Contributions

Plenary Lectures

P1 Bert Sakmann, Heidelberg (Roger-Eckert-Lecture)
Cortical microcircuits and their plasticity

P2 Jens Frahm, Göttingen
Magnetic resonance neuroimaging: From anatomy to function

P3 Michael Hagner, Berlin
Enchanted looms. On brains and the scientists in the 19th and 20th centuries

P4 Dietmar Kuhl, Hamburg
Learning about activity-dependent genes

P5 Nils Brose, Göttingen
Presynaptic plasticity: Dynamic regulation of neurotransmitter release at active zones

P6 Eckart O. Altenmüller, Hannover (Otto-Creutzfeld-Lecture)
From Laetoli to Carnegie: Musician's brains andnNeuroplasticity

P7 Fernando Nottebohm, New York, NY, USA (Ernst-Florey-Lecture)
Neuronal replacement in adult brain

P8 Silke Sachse, Berlin and New York, NY, USA
Odor processing in the honeybee antennal lobe

P9 Andreas Nieder, Cambridge, MA, USA and Tübingen
Of neurons and numbers: How the primate cortex encodes numerical information

Symposium: Adaptation: the psychophysicist's microelectrode

1 J. Zanker, Egham (UK)
Adaptation and contrast enhancement as universal coding strategies in the human visual system

2 M. Fahle, Bremen
Orientation bandwidth of perceptual learning

3 M. Bach and J. P. Maurer, Freiburg
Uncovering veridical human motion detectors in the EEG using "double adaptation"

4 M. W. Greenlee, Oldenburg
Gain control in visual cortex: Evidence from psychophysics and fMRI

5 D. A. Leopold, I. Bondar and N. K. Logothetis, Tübingen
Aftereffects with faces: Evidence for prototype referenced endoding of identity

Symposium: Juvenile hormone as a mediator of behavioural plasticity in adult insects

Symposium: Cytokines as mediators of neuroglial interactions

19 H. Siebert and W. Brück, Göttingen
Cytokines and proteases that influence sciatic nerve degeneration

20 G. Raivich, London (UK)
Cytotoxic potential of inflammation-associated cytokines in neuronal degeneration: Role of TNF-α and TGF-β1

21 C. Sommer, A. George and M. Schäfers, Würzburg
Expression and transport of tumor necrosis factor-α in peripheral nerve injury

22 J. Mey, K. Schrage and N. Jeliaznik, Aachen
Retinoic acid as a regulator of cytokine signaling in peripheral nerve regeneration

23 N. Jeliaznik and J. Mey, Aachen
Activation of retinoic acid signaling after sciatic nerve injury: Upregulation of cellular retinoid binding proteins

24 K. Schrage, V. Johann and J. Mey, Aachen
Cytokine expression in schwann cell primary cultures after retinoic acid treatment

25 H. Siebert and W. Brück, Göttingen
The influence of different cytokines and proteases on sciatic nerve degeneration – a study in different knockout mice

26 S. J. Haas, A. Ahrens, O. Schmitt and A. Wree, Rostock
Quinolinic acid lesions of the caudate putamen in the rat lead to an increase of Ciliary Neurotrophic Factor

Symposium: Transgenic animal models for neurodegenerative diseases

27 P. Kahle, München
Transgenic mouse models of α-synucleinopathies

28 H. Puccio
Mouse models of Friedreich's ataxia-models for oxidative stress

29 B. De Strooper, Leuven (Belgium)
Integral membrane proteolysis mediated by the presenilin/γ-secretase complex

30 F. L. Heppner and A. Aguzzi, Zurich (Switzerland)
Transgenic mouse models of prion disorders

31 R. Baumeister, München
Parkinson's and Alzheimer's disease in C. Elegans

32 U. Ueberham, E. Ueberham, R. Gebhardt and T. Arendt, Leipzig
Inducible neuronal expression of TGF-β1 in transgenic mice

33 E. Ramminger, U. Ueberham, A. G. Beck-Sickinger, R. Heumann and T. Arendt, Leipzig and Bochum
Altered expression of plasticity-related genes in syn-ras transgenic mice

34 S. Cambridge, B. Cürten and T. Bonhoeffer
A caged doxycycline analog for photoactivated gene expression with high spatiotemporal resolution

Symposium: Signal integration in dendrites

35 A. D. Reyes, New York, NY (USA)
Integration of Synaptic inputs: Summation in the subthreshold and suprathreshold ranges

36 J. Magee, New Orleans, LA (USA)
Regulation of local dendritic spike initiation and propagation in CA1 pyramidal neurons

37 M. Larkum, Heidelberg
Dendritic interactions in layer 2/3 neocortical pyramidal neurons

38 M. Häusser, London (UK)
Interactions of action potentials with somatic and dendritic ipsps

39 T. Berger, Bern (Switzerland)
Electrotonic separation of two spike initiation zones in layer 5 pyramidal cells of the somatosensory cortex: Role of the hyperpolarization-activated current I_h

40 G. Stuart and B. Kampa, Freiburg
Dendritic mechanisms involved in spike-timing dependent plasticity

41 N. Benhassine and T. Berger, Bern (Switzerland)
Biophysical properties and distribution of large-conductance calcium-dependent potassium channels in neocortical layer 5 pyramidal neurons

42 W. Senn, H.-R. Lüscher and M. E. Larkum, Bern (Switzerland) and Heidelberg
The gain of L5 pyramidal neurons is larger for distal than for somatic input

43 B. M. Kampa and G. J. Stuart, Freiburg
Dendritic mechanisms involved in spike-timing dependent plasticity

44 E. H. van den Burg, J. Bacelo, L. Gómez, J. Engelmann and K. Grant, Gif sur Yvette (France)
Inhibition of back-propagating spikes in a cerebellum-like sensory structure in the weakly electric fish Gnathonemus petersii*, by a general anaesthetic*

Symposium: Neuronal death and neuroprotection: The role of glial cells

45 C. Steinhäuser and G. Seifert, Bonn
Functional and molecular changes in astrocytes of human epileptic hippocampus: Relevance to seizure generation

46 J. A. Gorter, E. Hendriksen, E. van Vliet, F. Lopes da Silva and E. Aronica, Heemstede (The Netherlands) and Amsterdam (The Netherlands)
Molecular and immunocytochemical changes in macro- and microglia in a rat model of mesial temporal lobe epilepsy

47 P. Kofuji, Minneapolis, MN (USA)
Dystrophins, syntrophins and glial cell function in retina

48 D. Vivien and A. Buisson, Caen (France)
Can glial cells modulate excitotoxic neuronal injury?

49 A. Reichenbach and M. Francke, Leipzig
Muller cells protect neurons by transfer of glutathione, and by control of extracellular glutamate

50 T. Möller, Seattle, WA (USA)
Microglia: Friend or foe?

51 A. Wallraff, K. Hüttmann and C. Steinhäuser, Bonn
Complete lack of gap junctional coupling in a subpopulation of astrocytes, termed GluR cells, in the hippocampus

52 C. Krebs, H. Fernandes, C. Sheldon, A. Huxtable, A. El-Husseini, L. Raymond and K. Baimbridge, Bonn
Functional NMDA receptors in post-ischemia astrocytes – a possible synaptic target?

53 G. Seifert, K. Hüttmann, K. Matthias, C. Knott, G. Wilkin, C. Neusch, H. Lester and C. Steinhäuser, Bonn, London (UK), Göttingen and Pasadena, CA (USA)
Kir channels in the hippocampus: Different expression in distinct types of astrocytes and alterations under pathophysiological conditions

54 S. Walter, S. Kühl, Y. Liu, F. Mühlhäuser, K. Beyreuther and K. Faßbender, Göttingen
Alzheimer's disease. Beta-amyloid induces neuroinflammation via lipopolysaccharide receptor (CD14)

55 A. El Emmam Dief, C. Redecker, G. Metz, A. Aschoff, O. Witte, K. El Sabah and G. Jirikowski, Jena and Alexandria (Egypt)
Histochemical monitoring of apoptosis after cerebral ischemia and reperfusion in rat brain

56 M. Francke, I. Goczalik, D. Schwarze, M. Raap and A. Reichenbach, Leipzig
Neuronal glutathione supply by Müller cells during oxidative stress

57 T. Pannicke, B. Biedermann, O. Uckermann, M. Weick, A. Bringmann, S. Wolf, P. Wiedemann, E. Buse and A. Reichenbach, Leipzig and Münster
Physiological properties of retinal Müller glial cells from the monkey Macaca fascicularis – *comparison to human Müller cells*

Symposium: Drug addiction: Mechanisms and therapy

58 V. Höllt and S. Ammon, Magdeburg
Gene expression profile in rat brain after chronic morphine treatment

59 A. M. Zimmer, Bonn
Interactions between the opioid and cannabinoid systems

60 U. Schmitt and C. Hiemke, Mainz
Alcohol deprivation and the development of addiction

61 W. J. Schmidt, Tübingen
Glutamatergic mechanisms in addiction

62 W. Hauber, Stuttgart
Control of behaviour by reward-related stimuli

63 U. Havemann-Reinecke, Göttingen
Ecstasy, dependence and pharmacotherapy

Symposium: Precise timing in the brain: Linking neuronal activity and behaviour

64 M. Abeles, Jerusalem (Israel)
Scales for computational elements in the cortex

65 C. Mehring, M. Nawrot, J. Rickert, A. Riehle, S. Cardoso de Oliveira, E. Vaadia, A. Aertsen and S. Rotter, Freiburg, Marseille (France) and Jerusalem (Israel)
Decoding neuronal population activity associated with arm movements

66 J. Keating, Philadelphia, PA (USA)
Directional information flow in sensorimotor cortex during reaching as revealed by the gravitational transformation

67 P. Thier, P. W. Dicke, R. Haas, S. Barash and N. Catz, Tübingen and Rehovot (Israel)
Encoding of movement time by populations of cerebellar Purkinje cells

68 J. Hore, London, Ontario (Canada)
Precision and timing of motor output

69 V. Braitenberg, Tübingen
Spatio-temporal activity patterns as a key to cerebellar function

70 H. R. Dinse and I. van der Berg, Bochum
What is simultaneous? Tactile coactivation in human subjects reveals requirement for millisecond precision for induction of plastic changes

71 K. H. Kreikemeier, I. van den Berg and H. R. Dinse, Bochum
Effects of timing: Switching cortical map reorganization and perceptual learning

72 C. Oreja-Guevara, R. Gobbelé, F. Darvas, A. Dieckhoefer, H. Buchner and K. P. Hoffmann, Bochum, Aachen and Recklinghausen
Electrical source activity and interregional coherences of the human brain during visuomotor tasks

73 P. Ragert, B. Pleger, M. Tegenthoff, A.-F. Foerster, V. Nicolas and H. R. Dinse, Bochum
rTMS elicits tactile discrimination improvement and parallel plastic reorganization in human SI

74 B. Pleger, P. Ragert, A.-F. Förster, H. Dinse, V. Nicolas and M. Tegenthoff, Bochum
Functional magnetic resonance imaging of the human brain: Cortical reorganization controls somatosensory short-term learning

75 B. Hedwig, Cambridge (UK)
Coding of pattern recognition

76 D. Suchanek, F. Kuemmell, A. Aertsen and D. Heck, Freiburg
Investigating cortical network dynamics with combined intracellular and multi-electrode extracellular recordings

77 F. Sultan and D. Heck, Tübingen and Freiburg
Detection of sequences in the cerebellar cortex: Numerical estimate of the possible number of sequences represented

78 F. Sultan and S. Rotter, Tübingen and Freiburg
Simulating the cerebellar tidal-wave – variability in axonal conduction velocity constrains noisy inputs

Symposium: Ontogenetic cell death in the nervous system

79 G. Haase, C. Raoul, D. W. Cleveland, S. Corby, E. Buhler, C. E. Henderson and B. Pettmann, Marseill (France) and San Diego, CA (USA)
Motoneuron death through the Fas/NO pathway

80 A. Martin-Villalba, D. Demjen, S. Kleber, C. Zuliani and P. H. Krammer, Heidelberg
The role of CD95-Ligand in the nervous system

81 D. James, P. Parone, S. Montessuit, X. Roucou and J.-C. Martinou
Apoptosis: Confusing the mitochondria

82 A. I. Valenciano, R. Mayordomo, C. Segundo, F. Hallböök, F. De Pablo and E. J. De la Rosa, Madrid (Spain)
Regulation of programmed cell death during early neural development

83 K. Krieglstein
TGF-β is a key regulator in ontogenetic neuron death

84 N. Dünker and N. Schuster, Göttingen and Homburg
TGF-β modulated programmed cell death in the developing retina

85 E. Aden, Hamburg
Apoptosis determines the ontogenetic regression of the cave fish eye

86 J. Dorszewska and Z. Goncerzewicz, Poznan (Poland)
The oxidative DNA damage and repair (p53) in rat brain aging

Symposium: Arthropod neural and motor systems: From development to function and mechanics

87 M. Bate, Cambridge (UK)
Neural networks and behaviour in the Drosophila *embryo*

88 C. Consoulas, Athens (Greece)
A steroid-regulated gene is required for dendritic growth of motoneurons during metamorphosis of Drosophila melanogaster

89 C. Duch and T. Mentel, Berlin
Stage-specific activity patterns affect motoneuron structure during Manduca *metamorphosis*

90 R. Strauss, Würzburg
Control of Drosophila *walking and orientation behavior by functional subunits localized in different neuropils of the central brain*

91 H. Pflüger, T. Mentel, C. Duch, G. Wegener, H. Stypa and U. Müller, Berlin and Mainz
Fuel selection in locust flight muscle by the activity of neuromodulatory neurons

92 F.-O. Lehmann, Ulm
The control of vorticity in flying drosophila

93 S. Schönknecht, C. Duch, M. Scholz, J.-F. Evers and K. Obermayer
Multi compartment model *of developmental changes in dendritic shape during postembryonic motoneuron development*

94 P. Burkert and C. Duch, Berlin
Changes in CaM kinase II activity and localization correlate with distinct phases of motoneuron dendritic growth during Manduca *metamorphosis*

95 J. F. Evers, D. Münch and C. Duch, Berlin
Metric analysis of growth-cones during dendritic remodeling of an identified flight motoneuron in Manduca sexta

96 D. Münch, S. Schmitt, M. Scholz and H.-J. Pflüger, Berlin
Postembryonic growth of a first order interneuron in a developing sensory-motor circuit – A morphometric analysis

97 E. Heidel and H.-J. Pflüger, Berlin
Transient potassium currents in identified subtypes of octopaminergic dorsal unpaired median (DUM-) neurons isolated from locust thoracic ganglia

98 S. Schmitt, J. F. Evers, M. Scholz, K. Obermayer and C. Duch, Berlin
From voxels to model: Automatic reconstruction of neurons from confocal images

99 M. C. Göpfert, H. Stocker and D. Robert, Bristol (UK) and Zurich (Switzerland)
Genetically linked formations of sensory and accessory components in the auditory system of Drosophila

100 M. C. Göpfert and D. Robert, Bristol (UK)
Mechanical activity of Drosophila mechanosensory neurons

101 A. Prokop, G. M. Technau, B. Küppers, R. Löhr, K. Lüer, M. Mende and N. Sánchez-Soriano, Mainz
From the NMJ into the CNS – Synapse and circuit formation in fruitflies

102 S. Pick and R. Strauss, Würzburg
Towards the neuronal substrates underlying insect climbing behavior – a high-speed 3D-video analysis of normal and mutant fruit flies

Symposium: Adult neurogenesis

103 G. Kempermann, Berlin
From progenitor cells to new neurons in the adult brain: Possible functions for adult hippocampal neurogenesis

104 H. G. Kuhn, Regensburg
Adult neurogenesis: A balance of proliferation and cell death

105 J. Priller, Berlin
Engraftment of bone marrow-derived cells in the murine CNS

106 O. D. Wiestler, Bonn
Evidence for neurogenesis in human temporal lobe epilepsy

107 M. H. Höhn
How to track neurogenesis and stem cell activity in the adult brain

108 N. Braun, J. Sévigny, S. K. Mishra, S. C. Robson, S. W. Barth, R. Gerstberger, K. Hammer and H. Zimmermann, Frankfurt am Main, Sainte-Foy, Quebec (Canada), Boston, MA (USA), Karlsruhe and Gießen
The ecto-ATPase NTPDase2 is expressed in the germinal zones of the developing and adult rat brain

Symposium: Invasive recording from the human brain: Linking clinical applications with neurobiological research

109 A. K. Engel, C. K. E. Moll, C. Dohle, N. Allert, J. Voges, R. Lehrke, H.-J. Freund and V. Sturm, Hamburg, Jülich, Bonn and Köln
Microelectrode recordings from the human basal ganglia

110 P. Brown, M. Cassidy and D. Williams, London (UK)
Task-related coherence in Parkinson's disease

111 G. Fernandez, J. Fell, P. Klaver and C. E. Elger
Rhinal-hippocampal coupling during human memory formation

112 J.-P. Lachaux
Increase of high-frequency (>150 hz) intracranial EEG activity during face perception in humans

113 I. Fried, Los Angeles, CA (USA)
Dynamics of single neurons in the human medial temporal lobe during perception and memory tasks

Symposium: Longterm potentiaton and longterm depression of nociceptive CNS processing

114 T. Bliss, London (UK)
Long-term potentiation after 30 years - where do we stand?

115 A. J. Artola, Antwerp (Belgium)
Use-dependent synaptic plasticities in hippocampus and visual cortex

116 W. Zieglgänsberger, München
Extinction of aversive memory – a role for endocannabinoids?

117 J. Sandkühler, Vienna (Austria)
Synaptic LTP and LTD in spinal pathways

118 W. Magerl, Mainz
LTP- and LTD like plasticity of human pain perception

119 U. Ziemann, Frankfurt am Main
LTP-like plasticity in intact human motor cortex. Investigations with transcranial magnetic stimulation

120 A. J. Artola, Antwerp (Belgium)
Use-dependent synaptic plasticities in hippocampus and visual cortex

121 U. Ziemann, Frankfurt am Main
LTP-like plasticity in intact human motor cortex. Investigations with transcranial magnetic stimulation

122 A. Tappe, D. Hirlinger, J. Benrath and R. Kuner, Heidelberg
Selective induction of Homer1a in spinal neurons during pathological pain states via activation of NMDA receptors and Erk1/2

123 E. P. Kostyuk, Kiev (Ukraine)
Changes in neuronal calcium signalling during diabetic pathology

Symposium: Towards a molecular understanding of behavior

124 R. Harris-Warrick, Ithaca, NY (USA)
Potassium channels and the control of a rhythmic behavior

125 D. Parker and S. Bevan, Cambridge (UK)
Cellular and synaptic effects contributing to long-term neuropeptide-mediated modulation of a spinal cord locomotor network

126 R. Heinrich, Göttingen
Selection and control of behavior by intracellular signaling pathways in the insect brain

127 U. Müller, Berlin
Second messenger cascades: Major mediators of memory formation

128 E. A. Kravitz, S. Chen and A. Y. Lee, Boston, MA (USA)
Fighting Fruit Flies: A model system for the study of aggression

129 L. J. Young, Atlanta, GA (USA)
Vasopressin and social attachment in a monogamous mammal

131 K. Hoffmann, B. Wenzel, C. Günther, N. Elsner and R. Heinrich, Göttingen
The potency of acetylcholine to activate muscarinic receptors in the brain of grasshoppers

132 B. Wenzel, C. Günther, R. Lakes-Harlan, N. Elsner and R. Heinrich, Göttingen
Grasshopper acoustic communication behavior is inhibited by activation of the NO-/cGMP- signaling pathway in the brain

133 H. Rolf and M. Hoerner, Göttingen and Hong Kong SAR (China)
Fight or flight? Octopamine effects on the cricket escape pathway

134 M. Seifert, M. Gewecke and T. Roeder, Hamburg and Würzburg
The tyramine receptor of Caenorhabditis elegans

135 V. Dyakonova, A. Kruschinski and D. Sakharov, Moscow (Russian Federation)
To mate or to fight? Effects of flight on male-female relationships in cricket gryllus bimaculatus

136 U. Werner, K. Volkmann and H. Scholz, Würzburg
Functional dissection of the octopaminergic neurotransmitter system in ethanol tolerance in Drosophila

Symposium: Peptide co-transmitters in identified neurons

137 H. Dircksen, Bonn
Differential distributions and functions of orcokinins and orcomyotropin, novel partially co-localized peptides, in crayfish sensory, motor, interneuronal and neurosecretory cells

138 V. Fénelon, Y. Lefeuvre and P. Meyrand, Talence (France)
Ontogeny of modulatory systems

139 P. Skiebe, Berlin
Multiple members of a peptide family are present in single identified neurons

140 S. Kreissl, Konstanz
Antagonistic modulation of muscle contraction by two co-localised peptides

141 W. Stein, Ulm
Convergence and divergence of peptide cotransmitter actions: Functional consequences in a multifunctional network

142 A. A. Prinz, Waltham, MA (USA)
Dissecting and modeling the actions of neuromodulatory peptides on multiple targets in a network of identified neurons

143 V. Fenelon, Y. Lefeuvre and P. Meyrand, Talence (France)
Ontogeny of modulatory systems

Symposium: Early environmental programming: Molecular, neuroanatomical, neuroendocrine and behavioural effects

144 J. R. Seckl, Edinburgh (UK)
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145 A. K. Braun, C. Helmeke, P. Friedrich, T. Schwabe, W. Ovtscharoff Jr. and G. Poeggel, Magdeburg and Leipzig
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Ipsilateral motor pathway investigated by TMS and functional MRI in patients with recovered paralytic upper limb

975 J. Tan, I. Koepschall, K. Rohbock and M. Knipper, Tübingen
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977 M. H. Schwab, T. Fischer and C. Lai, Göttingen and La Jolla, CA (USA)
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980 M. Ergen, M. E. Erdal, T. Ergenoglu, H. Y. Keskin, H. Beydagi and T. Demiralp, Istanbul (Turkey) and Mersin (Turkey)
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981 A. Ihring, A. Borst and D. F. Reiff, Martinsried
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Parkinson in fruitfly, gene expression pattern changing during time?

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The expression of $GABA_A$- and AMPA-receptor mRNA in the primary motor cortex of patients with amyotrophic lateral sclerosis

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Expression of cell cycle regulators in the developing mouse brain

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Perineuronal nets potentially protect against oxidative stress

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Cell cycle and cell death in Alzheimer's disease: The role of cyclin B and cdk 1

996 J. Gerdelmann, J. Stieler and T. Arendt, Leipzig
The cdk5-activators $p25^{nck5a}$ and $p35^{nck5a}$ contribute to cell death of SH-SY5Y neuroblastoma cells

997 A. Riedel, R. Miettinen, I. Alafuzoff, H. Soininen and T. Arendt, Leipzig and Kuopio (Finland)
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Glioma cell migration along adult neurites – a new in-vitro model

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Endogenous antiepileptic processes are activated by epileptiform activity in a model nervous system

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Neuronal pacemaker potentials develop into epileptiform activity in model nervous systems

1001 A. Joschko and U. Altrup, Münster
Proteolytic enzymes trigger epileptogenic properties in a model nervous system

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1002 G. J. Feldmann, M. Bodemer, S. Poser, M. J. Schmerr and I. Zerr, Göttingen
Detection of abnormal prion protein in sporadic Creutzfeldt-Jakob disease (sCJD)

1003 I. Goczalik, I. Milenkovic, M. Raap, M. Weick, J. Heidmann, V. Enzmann, P. Wiedemann, A. Reichenbach and M. Francke, Leipzig
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Relationship between endogenous levels of cytokine mRNA in the striatum and anxiety-like behavior in the rat

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A link between the nervous system and the immune system in insects?

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1007 H. Lilienthal, A. Roth-Härer, A. Hack, H. Kaya and G. Winneke, Düsseldorf
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1008 E. M. Tousson, S. Adolf and H. Meissl, Frankfurt
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Direct molecular consequences of prolonged maternal deprivation on the hypothalmic-pituitary-adrenal axis

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Effects of a single social defeat on behavioural and neuroendocrine parameters in rats bred for extremes in anxiety

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Mice bred for high or low trait anxiety: A new murine model for emotionality

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1013 N. Dambeck, M. Wienemann, J. Weidemann, I. G. Meister, R. Töpper and B. Boroojerdi, Aachen and Hamburg
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Evidence for a spherical geometry of spatial color perception

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Reaction-time measurments show task-specific adaptations of mental finger representations in profesional pianists

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Recognition of object shape during active electrolocation in electric fish

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Duration but not level of intense inducer tones affect the loudness reduction of subsequent weaker tones

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1027 I. Hamann, O. Gleich, M. C. Kittel and J. Strutz, Regensburg and Regenburg
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1029 B. Mathes, S. J. Wood, G. W. Stuart, T. M. Proffitt, J.-A. Buchanan, W. J. Brewer, P. D. McGorry and C. Pantelis
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1030 A. Hodzic, A. A. Karim, R. Veit and B. Godde, Tübingen
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1031 S. Schreiber, J.-M. Fellous, P. Tiesinga and T. J. Sejnowski, Berlin, La Jolla, CA (USA) and Chapel Hill, NC (USA)
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1059 R. A. DuBois, N. Engel and G. Stuart, Canberra (Australia) and Freiburg
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1060 R. Ritz, R. Förster and A. V. M. Herz, Berlin
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1062 J. Mohr, A. Hess, M. Scholz and K. Obermayer, Berlin and Erlangen
Automatic extraction and visualization of functional information from autoradiographic brain image stacks

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1064 W. Horstmann, S. Lorenz and M. Egelhaaf, Bielefeld
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1065 J. P. Lindemann, N. Böddeker and M. Egelhaaf, Bielefeld
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1066 S. Kutluk, E. Bodur, S. Akar and A. N. Cokugras, Ankara (Turkey) and Darmstadt
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1067 V. Kloth, D. Lottrich and G. Nikkhah, Freiburg
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1070 A. Gennerich and D. Schild, Göttingen
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1071 C. Lohr and J. W. Deitmer
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1072 P. Blaesse, S. Löhrke and E. Friauf, Kaiserslautern
Single-cell electroporation of HEK293 cells and auditory brainstem slices

1073 M. Gabriel and U. T. Koch, Kaiserslautern
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1074 R. Pielot, M. Scholz, K. Obermayer, E. D. Gundelfinger and A. Hess, Magdeburg, Berlin and Erlangen
Volume warping of segmented brain data sets in autoradiographic imaging

1075 K. Kemnitz, Z. Petrasek and W. Zuschratter, Berlin and Magdeburg
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1076 W. Zuschratter, M. Jose, D. Dieterich, T. Dresbach, E. D. Gundelfinger, M. Kreutz, M. R. Kreutz and K. Kemnitz, Magdeburg and Berlin
Fluorescence lifetime imaging microspectroscopy of xFP-fused proteins in hippocampal cell cultures using ultra-low excitation levels and ultra-sensitive imaging detectors

1077 A. Kremper and R. Eckhorn, Marburg
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1078 M. J. Hinner, G. Hübener and P. Fromherz, Martinsried
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1080 N. Heim, O. Zapata-Hommer and O. Griesbeck, Martinsried
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1081 C. Pouzat, M. Delescluse and J. Diebolt, Paris (France) and Champs-sur-Marne (France)
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1082 C. Pouzat, M. Delescluse and J. Diebolt, Paris (France) and Champs-sur-Marne (France)
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1083 M. P. Bonomini, E. Fernandez and M. Bongard, San Juan de Alicante (Spain)
WAND – an open workbench for the analysis of neuronal data

1084 H. R. Polder, J. Planck, M. Weskamp, H. Terlau and M. Ferber, Tamm and Göttingen
An electronic device that measures series resistance during tevc recording in xenopus *oocytes*

1085 N. Birbaumer, B. Schoelkopf and H. Preissl, Tübingen
The Thought-Translation-Device (TTD): A brain-computer-interface for the completely paralyzed

1086 E. Horn, D. De Staerke, U. Friedrich, M. Viso and C. Dournon, Ulm, Toulouse (France), Bonn, Paris (France) and Vandoeuvre-les-Nancy (France)
Experiences from the german-french pupil outreach project biological research in space linked to the andromède mission to the international space station

1087 B. M. Schmitt, H.-R. Polder and H. Koepsell, Würzburg and Tamm
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1088 J. Kirsch, Heidelberg
Molecular determinants of inhibitory synapses

1089 H. Monyer, Heidelberg
Chemical and electrical synapses at GABAergic interneurones and significance thereof for synchronous activity

1090 H. Möhler, F. Crestani, J.-M. Fritschy and U. Rudolph, Zürich (Switzerland)
Functional relevance of $GABA_A$ receptor subtypes

1091 H. Lerche, Ulm
Impaired inhibition as a pathophysiological mechanism in idiopathic epilepsies

1092 H. Wolf, Ulm
Inhibition of muscles makes them move faster: Arthropod common inhibitors

1093 B. Grothe, Martinsried
Glycinergic inhibition in audition: Specific functions in temporal processing

1094 H. Neumann, Ulm
Extra-classical receptive field responses – Balanced inhibition and excitation in visual Gestalt organization

1095 M. Spitzer, Ulm
Inhibition and the prefrontal cortex: A central mechanism for cognitive and emotional control

1096 M. Egorova, G. Ehret and I. Vartanyan, Saint Petersburg (Russian Federation) and Ulm
Critical bandwidths and inhibition in auditory midbrain neurons of house mice

1097 M. Schmäh and H. Wolf, Ulm
Inhibitory motor neurones in the abdomen of locusts, stick insects and dragonflies are putative homologs

1098 M. Schmäh, H. Wolf and P. Bräunig, Ulm and Aachen
Specific inhibitory motor neurones supply body wall muscles in the locust prothorax

1099 C. Sommer, R. Kollmar, S. Schwab, M. Kiessling and W.-R. Schäbitz, Ulm and Heidelberg
Untitled

Satellite symposium: Molecular basis of neural repair mechanisms

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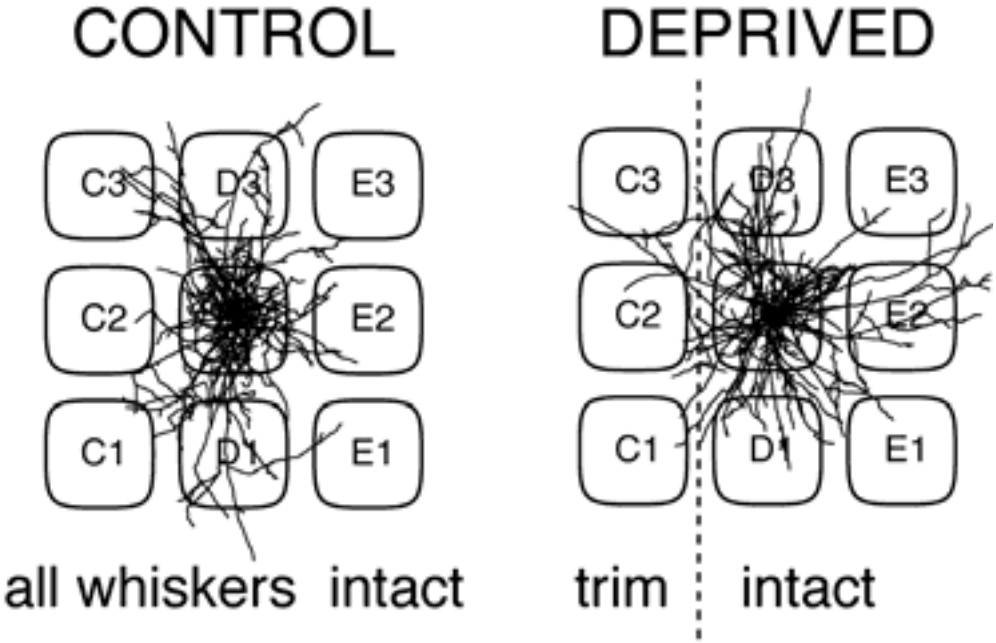
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Cortical microcircuits and their plasticity

P1

Bert Sakmann

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The brain operates by small electrical signals and if we want to understand mechanistically brain functions like the recognition of a sensory stimulus, the initiation of a limb movement or the storage and retrieval of memories we must measure and understand the time and space dependent pattern of electrical signals in different parts of the cerebral cortex. Despite the immense progress made during the last century in elucidating the physiology of cortical functions a number of basic questions remain unanswered. In particular the gap in understanding the properties of large ensembles, consisting of thousands of neurons for example those of a cortical column on the one hand and the properties of cortical microcircuits consisting of a small number of morphologically defined neurons located within and between cortical layers on the other hand is still rather broad.

Is a cortical function represented solely by the pattern of action potentials (AP) or by both subthreshold postsynaptic potentials (PSP) and APs? Which are those cell connections that are modifiable during “plastic” changes in the functional architecture of the cortex induced, for example, by the use or disuse of the cortex? What are the cellular mechanisms underlying the changes in the efficacy of synaptic connections?

The results from experiments made by combining fast optical imaging of the rodent somatosensory cortex, intracellular voltage recording *in vivo* and *in vitro* and the reconstruction of small circuits of connected neurons suggest that sensory stimuli are represented mostly by a pattern of subthreshold PSPs which, however, vary widely between different cortical layers in their extent and which change rapidly in time. Changes in the functional architecture of the cortex which can be induced by the use and disuse of the somato-sensory system during postnatal development are based on changes in the arborisation of L2/3 pyramid axons as well as changes in synaptic efficacy of the connections between pyramidal cells in layer 2/3. Such changes are triggered by coincident, suprathreshold activity in pre- and postsynaptic neurons involving both axonal and back-propagating dendritic APs.

P2 Magnetic resonance neuroimaging: from anatomy to function

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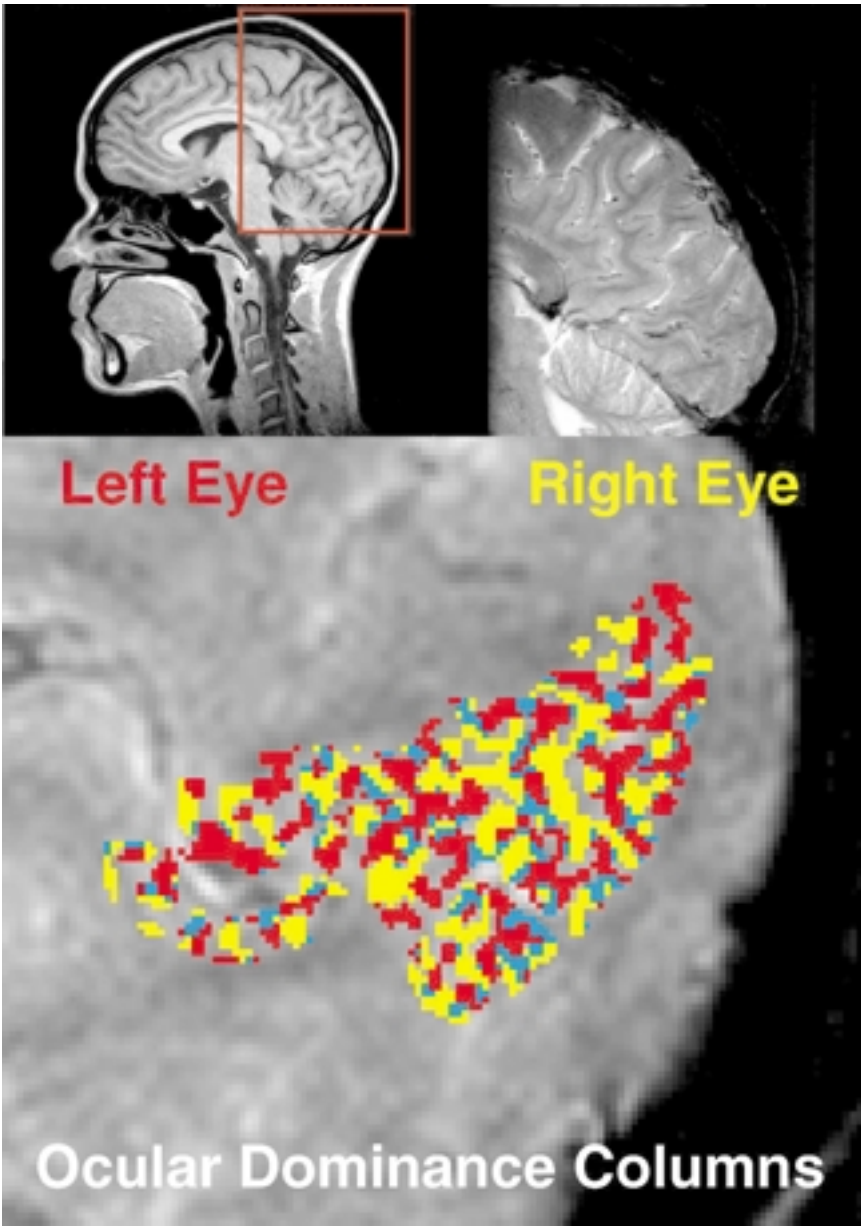
The tremendous success of magnetic resonance imaging (MRI) in medicine is based on its ability to visualize soft tissues at high spatial resolution and with excellent sensitivity to pathophysiologic alterations. This particularly applies to studies of the central nervous system. In contrast to techniques using ionizing radiation, MRI offers completely noninvasive examinations and therefore not only facilitates repeated follow-up examinations but also allows for studies of normal subjects. Apart from elementary contrast parameters such as the density, T1, and T2 relaxation time of water protons in brain tissue, additional insights are gained by using paramagnetic contrast agents.

During the past decade, the structural information obtainable by MRI has been complemented by an increasing number of techniques that attempt to characterize the functional state of the tissue rather than its mere morphologic appearance. A first example is magnetic resonance angiography (MRA) which discriminates between stationary and moving water protons and thus yields three-dimensional representations of the intracranial vascularization. More recently, MRI techniques have been developed which characterize the molecular mobility of intra- and extracellular tissue water based on diffusion properties. An important clinical application is the unsurpassed demarkation of ischemic lesions during acute stroke. Advanced methods exploit the full orientational information of the diffusion tensor and aim at an in vivo assessment of the axonal connectivity by `staining' white matter fiber tracks.

Even beyond such progress in neuroanatomic MRI, magnetic resonance spectroscopy (MRS) yields a neurochemical characterization of the cellular metabolism and composition. Localized proton MRS offers studies of both physiologic and pathophysiologic conditions with clinical applications ranging from differential diagnosis to monitoring of disease progression and therapy efficacy. Examples include neoplastic, inflammatory, metabolic, and degenerative brain disorders.

Most fascinating, functional MRI (fMRI) of brain activation, i.e. the visualization of cortical function, will gain major impact on system-oriented and clinical neuroscience. The approach has already started to revolutionize our understanding of the conscious human brain as it allows for a mapping of the neurofunctional organization across and within cortical systems from primary sensorimotor processing to cognition and emotion. This unique access to normal and aberrant brain function is further expected to improve and extend the diagnosis and management of a variety of neuropsychiatric disorders. The basis of the most commonly used fMRI technique is an activation-induced change in focal brain perfusion and oxygen metabolism. Variants provide access to submillimeter spatial resolution or subsecond temporal resolution and offer sensitivity to responses elicited by processing single brief cortical events.

The purpose of this lecture is to outline the overall potential of state-of-the-art magnetic resonance studies of the brain. Although most of the examples deal with applications to the human brain, additional comments will address the question to which degree MRI may contribute to imaging neuroscience of laboratory animals.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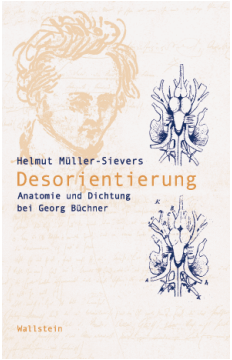

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Enchanted looms: on brains and scientists in the nineteenth and twentieth centuries. P3

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It is one of the remarkable characteristics of modern brain research since the nineteenth century that the brain became both a scientific object and a cultural organ loaded with values and symbols. Whereas in my book *Homo cerebralis. Der Wandel vom Seelenorgan zum Gehirn* (1997), I analysed the coming into being of the brain as the organ in which various mental qualities were localized, I shall now concentrate on the history of investigation of brains of extraordinary individuals: geniuses in the arts and sciences, and persons of special talent. The history of this subject is twofold: it comprises the establishment of a new scientific object — the ingenious brain — as well as idealized types that became crucial for the modern understanding of the scientist. Scientists, though usually employed by the state as professors, academicians, teachers, nevertheless remained extraordinary figures with specific characteristics. Their genius was supposed to be visible in their work and — post mortem — in their brains.

The interest in ingenious brains reaches back to the late eighteenth century, when physiognomicists and physical anthropologists established cerebral parameters for explaining intellectual differences among human beings. Reports about extraordinary brains were part of biographical sketches, often delivered in celebratory obituaries. The utilization of brains and skulls for cultural portraits of scientists was in accordance with the then available methods and parameters for investigating brains. These skills presaged the beginnings of modern elite brain research in the 1850s. In this decade, the older interest in brains and skulls of geniuses was transformed into a systematic and comparative exploration of brains of extraordinary persons. The first systematic investigations of ingenious brains were quite disappointing, but the years between the 1860s and the 1920s witnessed a remarkable number of dissections of this kind. On the one hand, these investigations were undertaken within the framework of localization theory, in particular the idea of cortical association centers, and of cytoarchitectonics. On the other hand, the interest in those brains was shaped by the cultural (self-)understanding of leading figures in society, including scientists, and by the eugenic idea of improving man. In conclusion, the collection and study of extraordinary brains were central to scientific culture in the first half of the twentieth century. Elite brain research came to an end for the time being with the emergence of the cybernetic approach in the neurosciences after World War II.

P4

Learning about Activity-Dependent Genes

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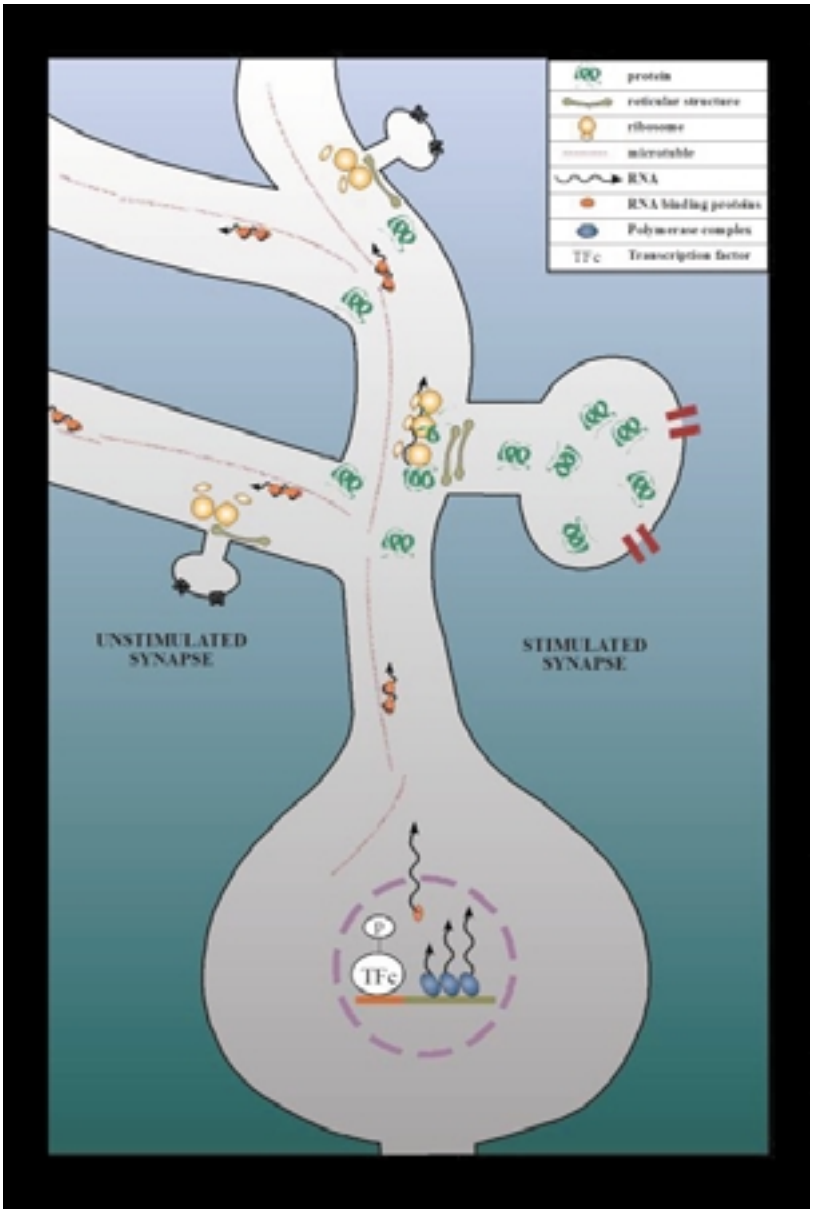
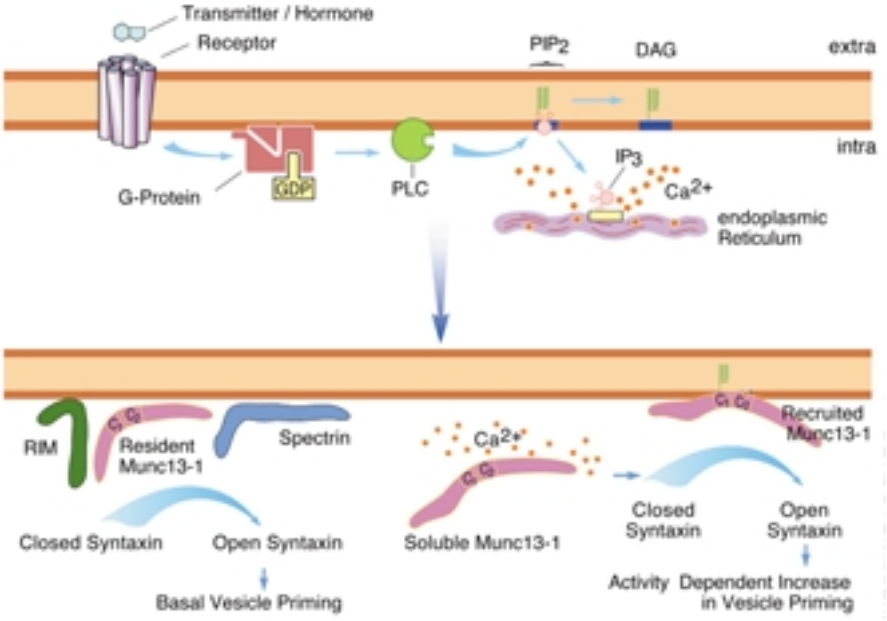
In animals and several cellular models of synaptic plasticity, long lasting changes in synaptic strength are dependent on gene transcription. Functional plasticity might therefore be achieved by activity-dependent changes in the expression of specific genes. Using subtractive cloning procedures we identified a number of genes that are induced in neurons of the hippocampus by plasticity producing neuronal activity. Analysis of their expression indicates a broad role for these genes in neuronal plasticity, including learning and memory. An example can be seen in the expression and distribution of Arg3.1/Arc mRNA and protein. Following LTP-producing stimulation Arg3.1/Arc mRNA synthesis and protein expression are dramatically induced. Moreover, following induction Arg3.1/Arc mRNA and protein are rapidly distributed throughout the dendritic arbor and can be specifically targeted to stimulated synapses. Biochemical analysis demonstrates that Arg3.1/Arc is present in the postsynaptic density (PSD), a highly organized biochemical machinery which, among others, anchors signaling molecules to ion channels. Furthermore, we find that Arg3.1/Arc is associated with the NMDA (N-methyl-D-aspartate) receptor complex. This association is not seen in animals that lack PSD95/SAP90, a protein that can directly bind to the NMDA-receptor. We have generated Arg3.1/Arc knockout animals, in which we deleted the complete gene. These animals exhibit striking and very specific deficits in the consolidation of LTP and memories. Thus, Arg3.1/Arc may act to maintain the functional integrity of the NMDA-receptor complex, and thereby coordinate the signaling pathways that control activity-dependent changes on the postsynaptic side.

While Arg3.1/Arc has a key role in memory formation, nothing is known about the mechanisms that govern Arg3.1/Arc mRNA transport into dendrites. To address this issue, we developed the Tri-hybrid Method for the in vivo reconstruction of specific RNA-protein interactions. Using this technique in a genetic screen we identified several clones that specifically interact with Arg3.1/Arc mRNA but not with perikaryal-control RNAs. One of these proteins we have named Zinki because it contains a domain of repeated zinc fingers required for the specific binding to Arg3.1/Arc mRNA. The expression of Zinki is predominantly dendritic. Moreover, we find that upon dendritic lamina specific stimulation, Zinki vacates those dendritic regions in which translation of the Arg3.1/Arc mRNA is enhanced suggesting that Zinki may control translation of the Arg3.1/Arc mRNA in this compartment.



A schematic illustration of synapse-specific modifications by local protein synthesis directed by dendritic Arg3.1/Arc mRNA. Trans-synaptic activation of transcription factors leads to induced transcription of this mRNA. Arg3.1/Arc transcripts are transported to the dendritic arbor, and enhanced translation of the transcripts takes place at trans-synaptically activated sites.


Transmitter / Hormone
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PIP2
DAG
extra
intra
G-Protein
GDP
PLC
IP3
Ca2+
endoplasmic
Reticulum
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Resident
Munc13-1
Spectrin
Closed Syntaxin
Open Syntaxin
Basal Vesicle Priming
Ca2+
Soluble Munc13-1
Recruited
Munc13-1
Closed
Syntaxin
Open
Syntaxin
Activity Dependent Increase
in Vesicle Priming

Presynaptic Plasticity: Dynamic Regulation of Neurotransmitter Release at Active Zones

P5

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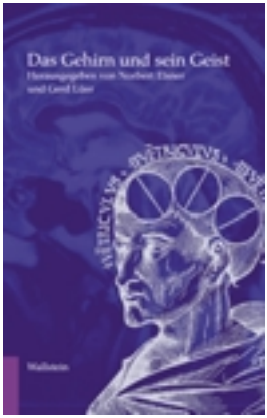
Munc13 proteins are essential synaptic vesicle priming factors that are thought to act by regulating the activity of the t-SNARE Syntaxin. In the absence of Munc13 mediated vesicle priming, synapses contain no fusion competent synaptic vesicles, which causes a complete arrest of spontaneous and evoked transmitter release.

The genome of rodents and primates contains three Munc13 genes, Munc13-1, -2, and -3, only two of which, Munc13-1 and -2, are expressed in hippocampal nerve cells. Depending on the Munc13 isoform present, synapses of hippocampal neurons are characterized by strikingly different short term synaptic plasticity: Munc13-1 containing synapses show synaptic depression during high frequency stimulation while Munc13-2 containing synapses show synaptic facilitation/augmentation under the same stimulation conditions.

The differences in Munc13 mediated short term plasticity, which are due to protein intrinsic characteristics of Munc13-1 and Munc13-2, are further modulated by two major second messenger pathways. First, Munc13-1 and Munc13-2 are the main functionally relevant targets of the diacylglycerol second messenger pathway. Interference with diacylglycerol binding to Munc13 proteins leads to dramatic changes in synaptic short term plasticity that are characterized by increases in synaptic depression in Munc13-1 dependent synapses and by a shift from facilitation/augmentation to synaptic depression in Munc13-2 dependent synapses. Second, Munc13-1 and Munc13-2 are regulated by Ca^{2+}/Calmodulin. As is the case with diacylglycerol binding, interference with Ca^{2+}/Calmodulin binding to Munc13-2 leads to a complete loss of the facilitating/augmenting phenotype in Munc13-2 dependent synapses.

Our data indicate that presynaptic short term plasticity in hippocampal nerve cells is initially regulated by the differential equipment of synapses with Munc13 priming proteins. A second level of regulation is provided by the modulation of Munc13 priming activity via the diacylglycerol and Ca^{2+}/Calmodulin second messenger pathways.



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From Laetoli to Carnegie: musicians‘ brains and neuroplasticity

P6

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Performing music at a professional level is probably the most complex of human accomplishments. Music, as a sensory stimulus, is highly complex and structured along several dimensions. Moreover, making music requires the integration of multimodal sensory and motor information and precise monitoring of the performance via auditory feedback (Fig. 1).

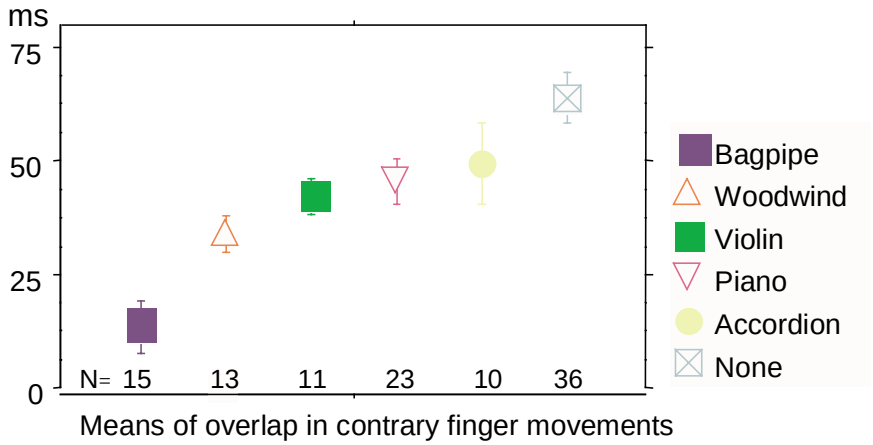

Figure 1. Behavioural adaptations: The role of auditory feedback in a sensory-motor transfer task. Overall averages of a test of synchrony in different groups of professional musicians. The task was to avoid any overlap whilst touching with one finger and synchronously releasing with another finger a Morse key system. The pipers clearly have the least errors, followed by woodwind players. The results demonstrate that motor control in musicians is specifically guided by auditory feedback since avoiding overlap is critical in any pipes and woodwind instruments, but not in keyboards and the accordion (bars = ±1 S.E.M.)

In the context of western classical music, musicians are forced to reproduce highly controlled movements almost perfectly with a high reliability. These specialized sensory-motor skills require extensive training periods over many years, starting in early infancy and passing through stages of increasing physical and strategic complexities. The superior skills of musicians are mirrored in plastic adaptations of the brain on different time scales (Fig. 2, for a review, see: Münte et al. 2002). At one extreme, years of musical experience, especially in those musicians who begin training early in life, might lead to an increase in gray and white matter volume in several brain regions,

including sensory-motor and auditory areas, the cerebellum and the anterior portion of the corpus callosum. These anatomical alterations appear to be confined to a critical period. The fact that in several of the studies a correlation was found between the extent of the anatomical differences and the age at which the musical training commenced strongly argues against the possibility that these differences are pre-existing and the cause for rather than the result of practicing music. At the other extreme, several minutes of training can induce changes in the recruitment of auditory or motor cortex areas, or establish auditory-sensory-motor coupling (Bangert et al. 2001).

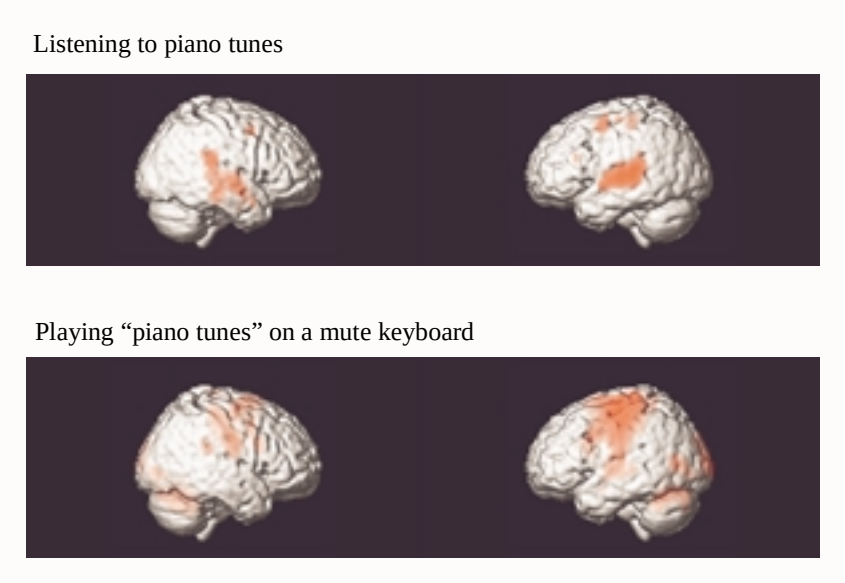

Figure 2.Central nervous adaptations: Auditory-sensory-motor co-representation in a professional pianist investigated with fMRI. Listening to piano tunes activates in addition to auditory brain regions sensory-motor areas; playing on a mute keyboard activates additionally mainly left auditory areas. (Figure courtesy to Dr. Marc. Bangert and Dr. Thomas Peschel, modified from Bangert and Altenmüller 2003

There is a dark side to the increasing specialisation and prolonged training of modern musicians, namely loss of control and degradation of skilled hand movements, a disorder referred to as musicians’ cramp or focal dystonia (Fig. 3). The first historical record, from 1830, appears in the diaries of the ambitious pianist and composer Robert Schumann. As was probably the case for Schumann, prolonged practice and pain syndromes due to overuse can precipitate dystonia, which is developed by about 1% of professional musicians and usually ends their career (Lim et al. 2001). Neuroimaging studies point to dysfunctional (or maladaptive) neuroplasticity as its cause.

Fig. 3. Dysfunctional Plasticity: Focal dystonia in a pianist. Involuntary flexion of middle, fourth and fifth fingers whilst attempting to play a C-major scale with the right hand.

Support for this theory comes from a functional brain imaging study performed in musicians with focal dystonia. Compared to healthy musicians, the dystonics showed a fusion of the digital representations in the somatosensory cortex, reflected in the decreased distance between the representation of the index finger and the little finger when compared to healthy control musicians (Elbert et al. 1998). Such a fusion and blurring of receptive fields of the digits may well result in a loss of control, since skilled motor actions are necessarily bound to intact somatosensory feedback input. Considering 1) the historical advent of the disorder in the nineteenth century with rapidly increasing technical demands imposed on musicians, 2) the epidemiological data with cumulative life practice time as a risk factor, and 3) neurobiological findings of the blurring of hand representations, one is tempted to state that focal dystonia finally marks the natural limits of a process of refinement of manual dexterity over a million years.

In summary, music making is an excellent model in which to study the effects of neuroplasticity in the auditory and the sensory-motor domains. It seems to be one of the most powerful stimuli to drive plastic changes in the central nervous system. The study of professional musicians might allow to differentiate the contributions of experience or training from those of genetic predisposition. Studying focal dystonia finally, can illustrate the effects of dysfunctional plasticity due to overuse. An important question arises from the investigations presented here. One has to bear in mind that essential to music is to elicit strong emotional reactions. Shivers down the spine, tears in the eyes, a lump in the throat whilst listening to music are accompanied by the activation of a brain network which is involved in reward, emotion and motivation. Further research is required to show whether activity in these areas is mediating the powerful effects on neural plasticity.

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P7

Neuronal Replacement in Adult Brain

Fernando Nottebohm,

The Rockefeller University, New York, NY, USA

This talk was originally intended as a contribution to the 28th Göttingen Neurobiology Conference in 2001. Unfortunately, Professor Nottebohm was unable to attend the conference at that time.

A full paper has been published in:

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The Neuroscienses at the Turn of The Century
Proceedings of the 4th Metting of the German Neuroscience Society 2001, Volume I
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P8

Odor processing in the honeybee antennal lobe

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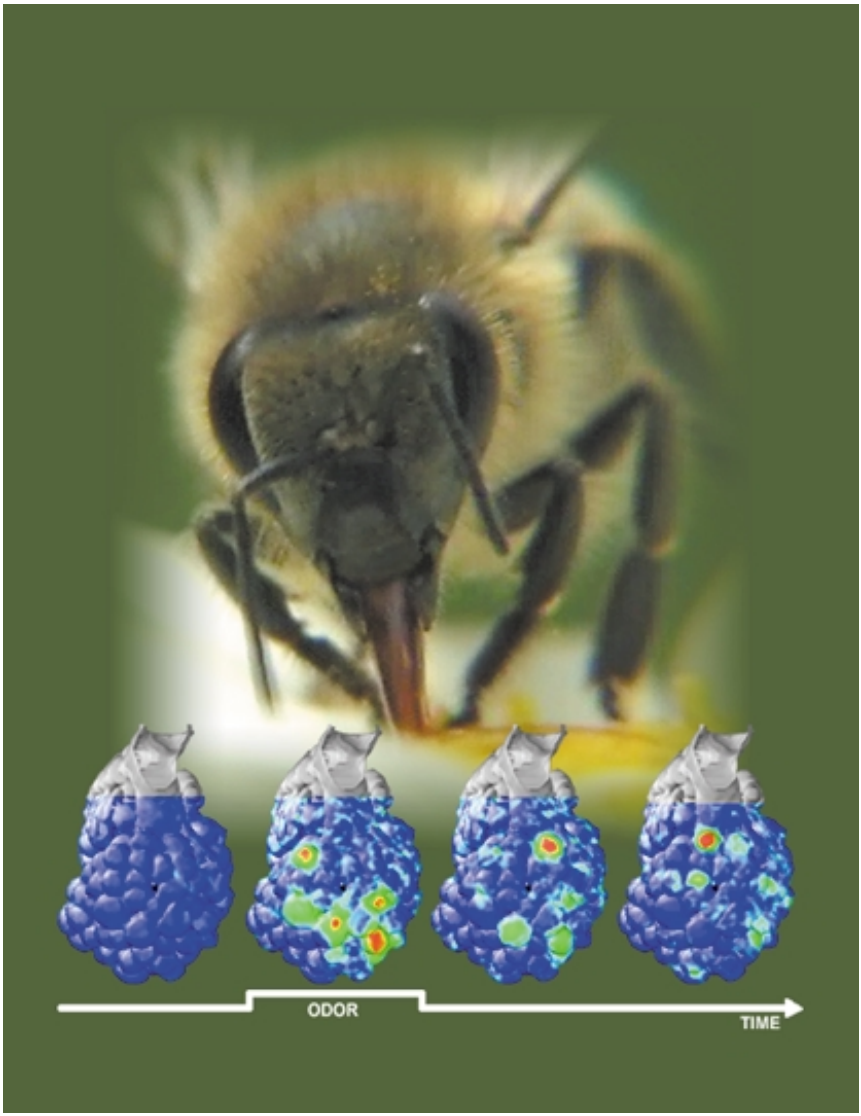
[2] University of California, Riverside, USA

The most important olfactory brain center - the antennal lobe (AL) in insects or the olfactory bulb in vertebrates - is a notable example of a neural network. The functional units of the AL are the olfactory glomeruli, which collect information from receptor neurons and are interconnected by local neurons. Odors evoke combinatorial patterns of activated glomeruli, which contain sufficient information to identify the stimulus. However, how the brain reads this code is not yet understood. While physiological properties of the input - the olfactory receptor neurons – have become clearer, the operation of the network itself remains cryptic. Complex inhibitory circuits are involved in the processing of odors to shape the olfactory code. Optical imaging techniques allow measuring simultaneously the activity of many identified glomeruli. This approach will become more powerful with increasing selectivity of the imaged neurons. We have therefore developed a novel calcium imaging technique to simultaneously but separately measure the receptor neuron input and the projection neuron output responses to olfactory stimulation of identified glomeruli.

We tested different odors spanning 7 log units of concentration. Increasing odor concentration led to stronger responses and more glomeruli being excited over the AL. Stimulus intensity may thus be encoded as overall excitation. Dose-response functions of the most-responsive glomeruli were sigmoidal and comprised dynamic ranges of 3-4 log units for both input and output neurons. Glomeruli with weak and intermediate responses in the input had reduced responses in the output, leading to lower numbers of activated glomeruli and contrast-enhanced odor representations. Odors can be separated to much lower concentrations in the output neurons than in the input. We also investigated the functional role of inhibitory connections by applying the two inhibitory transmitters GABA and histamine and the chloride-channel blocker picrotoxin. The results show the presence of two independent inhibitory networks: one is GABAergic and modulates overall AL activity, the other is PTX-insensitive and glomerulus-specific. Inhibitory connections of the latter network selectively inhibit glomeruli with overlapping response profiles, in a way akin to ‘lateral’ inhibition in other sensory systems. Taken together, the AL network optimizes odor representation by expanding the dynamic concentration range, improving concentration-invariance while maintaining intensity information, and increasing stimulus-uniqueness.


ODOR
TIME

P9 Of neurons and numbers: How the primate cortex encodes numerical information

Andreas Nieder

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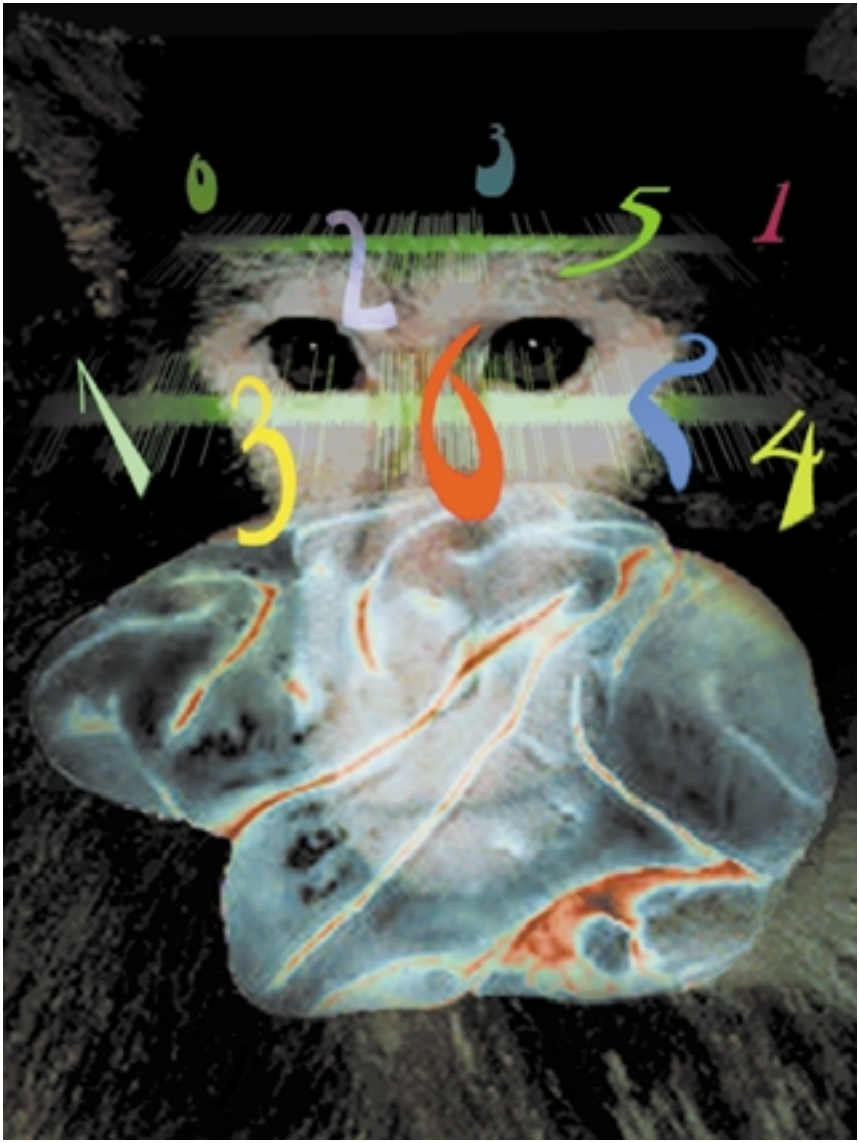
Evolution has endowed our brains and those of many animal species with simple numerical abilities. Social animals such as primates can make decisions like fight or flee by judging the relative number of friends versus foes and in foraging, choosing a larger alternative can contribute to survival. Non-verbal numerical competence in animals supports the hypothesis of phylogenetic precursor system(s) for higher, verbal-based numerical abilities in adult humans. Thus, investigating the neural mechanisms that give rise to numerical competence in animals may help to elucidate higher mathematical abilities only found in humans. Single-unit activity was recorded from the prefrontal (PFC), posterior parietal (PPC), and anterior inferior temporal (aITC) cortices of two monkeys trained on a delayed match-to-numerosity task in which they judged whether two successive displays contained the same small number of items (1 to 5). After training with displays of dots of various sizes and locations, the monkeys instantly generalized across new stimulus sets that controlled for low-level visual features (total area and circumference equated across different quantities, low vs. high dot density, dots arranged in lines or polygons, and dots replaced with other shapes). About one third of the neurons in the PFC were tuned for numerosity during stimulus presentation and memorization. Like the monkeys, neurons generalized across stimulus sets, suggesting that they underlie an abstract representation of quantity. When monkeys made judgment errors, neural activity for the preferred quantity was significantly reduced, implying that PFC neural activity contributed to behavioral assessment. The tuning curves formed overlapping filter functions whose properties can explain why discrimination between two numerosities improves with increasing numerical distance (*numerical distance effect)* and why discrimination of two quantities with equal numerical distance worsens as their numerical size increases (*numerical magnitude effect*). The tuning curves, which may be regarded as neuronal numerical representations, were best described by a non-linearly compressed scale, as postulated by the Weber-Fechner law or Stevens' law for psychophysical/sensory magnitudes. These results suggest that certain cognitive (i.e., numerosity) and perceptual/sensory representations share the same fundamental mechanisms and neural coding schemes. In contrast to the findings in PFC, a relative small proportion of numerosity-selective neurons were found in visual areas PPC and aITC, which provide the PFC with visual input. Only 4 to 11 % of the cells in any subregion of PPC discharged as a function of numerosity. Changes of visual features in the displays had a neglectible impact on parietal neurons` firing rate. In aITC, however, about half of the neurons that seemed to encode numerical information were sensitive to the visual features of the displays, resulting in only 10 % of the neurons that showed abstract numerosity selectivity. Together, these data show for the first time single neurons in the behaving primate brain that signal abstract numerical information in visual displays. The comparison between PFC, PPC and aITC suggests a dominate role of primate PFC in extracting and memorizing numerical information.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Introductory Remarks to Symposium 1

Adaptation: the psychophysicist's microelectrode

Nikolaus Troje and Michael Bach

Adaptation is a very general and basic phenomenon in biological information processing, covering a broad range from gain control to „fatigue“. Adaptation provides an active mechanism for efficient data compression by removal of redundancy: encoding changes of properties rather than the properties themselves allows the visual system to acquire, transmit, process and store information in a highly economical manner while minimising losses. However, besides its functional significance, adaptation has also proven to be a valuable scientific instrument to non-invasively investigative, characterize and isolate sensory information processing pathways.

In the visual domain, adaptation has traditionally been used mainly to study early visual processing. During the last few years, however, it has become evident that adaptation and corresponding after-effects also play a major role in high-level cognitive processing and that it can be employed to study phenomena such as face recognition or biological motion perception. In this symposium, we want to trace this development spanning the whole range between low-level vision and high-level cognitive processes, on the one hand, while emphasizing the dualistic nature of adaptation as a neural mechanism and as an investigative tool, on the other hand.

J. Zanker will open the series of presentations by providing a general introduction into the concepts of spatio-temporal visual signal coding that lead to the phenomena of after-effects in time as well as to simultaneous contrast enhancement in space. Using the example of motion boundaries in time and space he will illustrate this point in more detail by comparing results from a computational motion detection model to psychophysical observations.

The next two talks will provide illustrative examples of the use of adaptation for probing the properties of low level visual filters. In the contribution of M. Fahle selective adaptation is used as a tool to study the effects of perceptual learning on the characteristics of orientation selective visual filters. M. Bach uses a complex double adaptation paradigm to isolate direction specific motion responses in VEPs from direction unspecific flicker responses.

M. Greenlee's contribution is particularly interesting because he shows that contrast gain control, a mechanism that implements adaptation to varying light intensities, itself can be highly adaptive, therefore demonstrating „second order adaptation“ in the visual system.

In the last two contributions it is shown that adaptation is not only a low-level visual phenomenon. D. Leopold presents data on aftereffects in face recognition and N. Troje finds similar effects for biological motion perception.

1 Adaptation and Contrast Enhancement as Universal Coding Strategies in the Human Visual System

Johannes Zanker

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Sensory adaptation and after-effects are amazingly common phenomena that can be observed in a huge range of stimulus modalities. Furthermore, they are remarkably stable in an evolutionary sense, being found in the most simple as well as in the most highly developed sensory systems. This suggests that adaptation reflects the function of a very fundamental coding strategy in nervous systems. An abstract analysis of such mechanisms indeed reveals that adaptation enables an information processing system to transmit crucial stimulus components while removing redundant components.

Data compression by removal of redundancy is achieved by the encoding changes of properties rather than the properties themselves. This allows the visual system, for example, to acquire, transmit, process and store huge amounts of data in a highly economical manner while minimising information losses. From this point of view it is not surprising to find analogue phenomena in the spatial and temporal domain - visual signal encoding in space involves mechanisms that enhance stimulus contrast across feature boundaries and minimises neural responses for regions in which a stimulus feature does not change. Such spatial mechanisms lead to a number of (for instance, brightness) illusions that usually are referred to as ‘simultaneous contrast’, as opposed to ‘successive contrast’ in the temporal domain. Again, it is clear that spatial contrast enhancement mechanisms are found for the full range of sensory modalities and are universal across the animal kingdom.

These principles of signal encoding are illustrated with examples from visual motion processing, combining psychophysical observations with the results of computational modelling. Motion boundaries in time and space are crucial for a wide variety of behavioural and perceptual tasks and bring about an interesting computational challenge. The intricate balance of integrating and separating local signals that is required for detecting such spatial boundaries becomes apparent when studying motion-based segmentation and the perception of motion transparency. The human visual system appears to contain special units (‘channels’) dedicated to the processing of motion boundaries. Similarly, in the temporal domain the changes of velocity are the most significant events, which can be encoded by means of adaptation mechanisms, leading to some of the best known perceptual illusions - usually referred to as ‘motion after-effect’ or ‘waterfall illusion’. Because motion processing essentially involves temporal filters, in this particular instance it needs to be distinguished between inherent dynamic properties of the elementary process on one hand, and specific extensions of the basic mechanisms on the other hand, which can generate more general adaptation properties and spatial interactions (such as adaptive changes in time constants or push-pull mechanisms combined with temporal filters).

Orientation Bandwidth of Perceptual Learning 2

Manfred Fahle

Human Neurobiology, University of Bremen, Argonnenstr. 3, 28211 Bremen, Germany

Perceptual learning improves, through training, performance even in very basic perceptual tasks. In order to investigate the neuronal mechanisms underlying perceptual learning, psychophysical studies on the orientation selectivity of learning were performed.

One of the first stages of visual scene analysis is subserved by orientation selective visual filters such as the complex cells described in primate visual cortex. The bandwidth of these orientation selective filters has been measured previously by adaptation and masking experiments in humans to be around 10° to 20° at half width. These values correspond roughly to the orientation bandwidth of single neurones in, e.g., monkey visual cortex.

If perceptual learning would improve performance by sharpening these filters, it should show a similar orientation bandwidth. Five groups of 6 to 12 observers each trained a vernier discrimination task, thereby improving their ability to discriminate between small displacements between the vernier elements to the right versus to the left, to thresholds below the spacing of foveal photoreceptors. After one hour, the stimulus was rotated by 90, 45, 20, 10, or 4 degrees.

While there was no transfer of improvement between stimulus orientation of 90, 45, 20 and 10 degrees, there was virtually complete transfer at 4 degrees orientation difference. The resulting bandwidth of orientation selective filters is clearly below the values obtained from both masking studies using grating stimuli and single cell responses of monkey (primary) visual cortex. Hence, perceptual learning cannot primarily rely on sharpening the bandwidth of early orientation selective filters, but has to rely on more sophisticated neuronal mechanisms, probably involving top-down control.

Uncovering Veridical Human Motion Detectors in the EEG using “Double Adaptation” 3

Michael Bach and J. Peter Maurer

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Onset of visual motion evokes a component in the EEG, the motion VEP. Testing for its motion specificity with a direction-specific adaptation paradigm, previous work found that less than 50% of the motion-onset VEP represents veridical motion detection. Preadaptation of flicker detectors can help to isolate motion-specific mechanisms in the VEP: Using random-dot kinematograms with limited dot lifetimes we studied direction specificity after motion adaptation in eight subjects. Mean dot lifetimes were varied from 40 ms to infinite. With the longest dot lifetime, motion adaptation reduced the VEP amplitude to 35% (adapted direction) resp. 50% (opposite direction). With the shortest dot lifetime, motion adaptation reduced the amplitude to 55% (adapted direction) resp. 70% (opposite direction); the absolute VEP amplitudes were reduced to about 50%. These findings suggest that random-dot kinematograms with short dot lifetimes would

improve the investigation of human motion processing, be it in electrophysiology or other fields: While preadapting flicker detectors, such stimuli still evoke a sizable response, of which 70% is motion-specific.

4 Gain Control in Visual Cortex: Evidence From Psychophysics and fMRI

Mark W. Greenlee

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Contrast gain defines the relationship between the change in neural response and the change in stimulus contrast. We show how dynamic changes in contrast gain can lead to perceptual benefits for suprathreshold discriminations. Prior adaptation to spatial patterns changes the gain of the neurons. These changes can occur rapidly, while recovery may take place at a much slower rate. We review evidence for gain control from psychophysics, EEG and fMRI.

5 Aftereffects with faces: Evidence for prototype referenced encoding of identity

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We examined how the perception of face identity was influenced by prior exposure to a different face. We found that, following a few seconds of adaptation to one face, the identity of a second face was systematically misperceived. This identity aftereffect modulated perception in a manner consistent with a shift along a particular trajectory in multidimensional `face space'. This trajectory passed through the central tendency of all faces, and its direction thus defined a particular identity. The results suggested that the visual system considers the average prototype face to be a reference point in its representation of faces, and led us to speculate that neural decoding of faces is a fundamentally comparative process. Such a scheme might constitute a fast and economical storage strategy for the brain to contend with a myriad of very similar shapes. With the aim of investigating this hypothesis more directly by neurophysiological methods, we recently trained a monkey to perform the same task, again with human faces. We found that, while the monkey's identification thresholds were slightly higher than the mean threshold for humans, his perception was affected by adaptation in exactly the same way as that of the human subjects. Finally I will report our initial neurophysiological findings, obtained using implanted microelectrode bundles in the inferotemporal cortex.

High-level Aftereffects in Biological Motion Perception

6

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The human visual system shows an impressive sensitivity to subtleties in animate motion patterns carrying biologically relevant information. Frontal views of biological motion point-light walkers can be classified with respect to the gender of the walker with high accuracy. Here, we document pronounced adaptation effects that alter the perceived gender of a point-light walker.

Stimuli were generated using a morphing technique which provides smooth transitions along a linear discriminant function classifying a set of 80 walkers according to their sex[1]. Thirteen different walkers were sampled along the male-female walking axis covering a total range of 7 standard deviations of the walker distribution. In a first experiment we determined the location of a perceptually neutral walker on the male-female axis. Five different presentation times between 350 and 7000 ms were used. Using a two-alternative-fourced-choice procedure, subjects had to indicate for each display, whether it showed a man or a woman. The data were fitted by logistic psychometric functions. A morph half way between an average male and an average female walker was rated to be male. To obtain a perceptually neutral walker about one standard deviation of femaleness has to be added. Those results were independent of the presentation time.

In a second experiment, observers were first presented with 7000 ms point-light displays of either an exaggerated male walker, an exaggerated female walker or a perceptually neutral walker. After this adaptation period, they were tested with short presentations of walkers sampled along the male-female walking axis. The presentation time of the test stimulus was either 350, 700, or 1400 ms. Adaptation results in a pronounced shift of perceived gender of the test stimulus. A neutral walker is perceived to be female after adaptation with the exaggerated male and male after adaptation with the exaggerated female walker.

Our data demonstrate that adaptation can occur not only within low-level vision processes but also at high-level information processing stages. In the case of biological motion perception the aftereffects are affecting stages at which the complex information from the series of single moving light-dots is integrated into a coherent percept of walking person.

This research is funded by the Volkswagen Foundation.

[1]: Troje, N. F. (2002). Decomposing biological motion: A framework for analysis and synthesis of human gait patterns. Journal of Vision, 2:371-387, http://journalofvision.org/2/5/2/, DOI 10.1167/2.5.2.

7 Stereo Disparity and Chromatic Adaptation

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A central issue for understanding colour appearance in complex scenes is the interaction between colour and geometrical features of the scene. In a previous study we reported that the early time course (0.2- 5 s) of mid-spectral chromatic adaptation is accelerated by a cortical mechanism, which responds specifically to the spatial frequency and orientation of the adaptation pattern (Werner & Sharpe, 2002). Such interactions can be useful for the perceptual organization of a scene, which has been shown to influence colour appearance (Schirillo & Shevell, 2000; Bloj et al., 1999). Furthermore, it has been shown that colour constancy is increased if stereoscopic cues are present (Yang & Shevell, 2002). How depth cues influence colour constancy is, however, an unresolved question.

The purpose of the present study was to investigate whether the spatial structure of the background exerts an influence on chromatic adaptation across depth planes. The stimuli were produced on a calibrated monitor and consisted of two identical, segmented background patterns (each 10.6° x 11°, for the reference condition achromatic (u'=0.197, v'=0.468; luminance mean= 19.3cd/m2). The patterns were binocularly fused and the perceived depth relation between the central test-field and the background was produced by introducing retinal disparity. Chromatic adaptation was measured for the transition from D65 adaptation to a 5 s green adaptation-light (chromaticities chosen from the cardinal axes after Krauskopf et al., 1982) in an equiluminant plane in colour space. The effect of chromatic adaptation was measured by a hue cancellation technique for the achromatic appearance of the central test-patch. Adaptation was measured with (a) the test-field and its background perceived at the same depth plane (zero disparity), (b) at different depth planes (crossed disparity 1.9 min arc - 25 min arc) and (c) with test-field being perceived in the same plane as adjacent patches, but in a different depth plane as the remaining background (same retinal disparity as in (a)). It was found that the influence of the background on adaptation of the test-field decreased with increasing disparity. For the same disparity of test and background, however, adaptation was increased when patches adjacent to the test-patch were perceived in the same depth plane as the test (condition c). It is concluded, therefore, that chromatic adaptation is not only specifically influenced by the spatial frequency and orientation of the background, but also by its perceived depth plane. It is proposed that this mechanism supports the perceptual organisation and thus better colour constancy in 3D scenes. Supported by the University of Tübingen fortüne programme (#1059-0-0) and DFG (WE1710/1-1).

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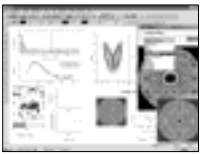

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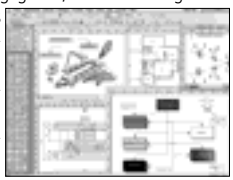

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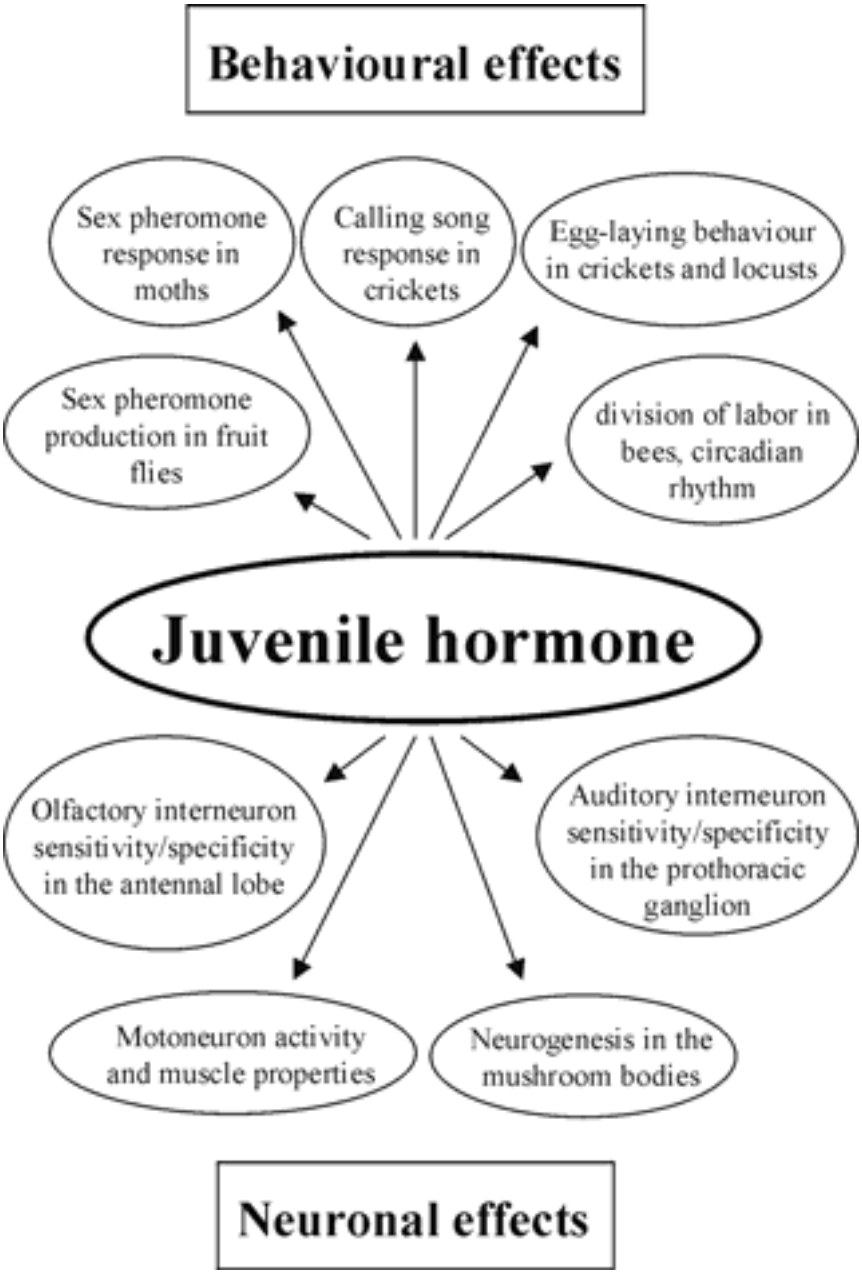
Behavioural effects
Sex pheromone response in moths
Calling song response in crickets
Egg-laying behaviour in crickets and locusts
Sex pheromone production in fruit flies
division of labor in bees, circadian rhythm
Juvenile hormone
Olfactory interneuron sensitivity/specificity in the antennal lobe
Auditory interneuron sensitivity/specificity in the prothoracic ganglion
Motoneuron activity and muscle properties
Neurogenesis in the mushroom bodies
Neuronal effects

Introductory Remarks to Symposium 2

Juvenile hormone as a mediator of behavioural plasticity in adult insects

Uwe Rose and Sylvia Anton

Since its discovery by Wigglesworth in 1934, Juvenile hormone (JH) has been known as an important regulator of insect developmental processes. In recent years, JH has also been pointed out as one of the major hormones regulating reproductive development in adult insects. In addition to its effect on the maturation of reproductive organs, it also influences the morphology and function of the nervous and muscular system, thereby regulating sexual and other age-related behaviour. This symposium will highlight JH-regulated behaviour and possible mechanisms of JH action in different insect species.

In general, an increase of JH biosynthesis during early adult life has been shown in all insect species investigated and the link of the observed behaviours with JH levels has been made through manipulation of these levels either by allatectomy or by injection of JH or JH analogs. Effects of JH have been shown to be reversible, demonstrating the plasticity of hormone-mediated behaviour.

At the beginning of this symposium P.E.A. Teal and Y. Gomez-Simuta will discuss the pivotal role of JH for the development and coordination of sexual signalling in Tephritid fly species. The ability to perceive signals from possible mating partners is important for a successful mate finding and the talks by C. Gadenne and J. Stout deal with JH-dependent changes in the sensitivity of two different sensory systems. In male noctuid moths, C. Gadenne showed that behavioural sensitivity and sensitivity of central olfactory neurons to female-emitted sex pheromone increase with age and JH level. J. Stout will show that the attraction of female crickets by calling songs produced by conspecific males changes with JH level. Plasticity and development of the female phonotactic behaviour can be understood by changes in the response properties of prothoracic auditory neurons.

In some insect species egg-laying behaviour is triggered by elevated JH levels. U. Rose will talk about the locust motor system that undergoes JH-dependent morphological and functional remodelling which are a pre-requisite for a successful egglaying behaviour. In the mushroom bodies of crickets, JH has been shown to stimulate neurogenesis and M. Cayre will discuss the question whether these newly generated neurons play a role in the maturation of egg-laying behaviour.

In honey bees age-related division of labour depends on JH hemolymph titers. Division of labour is also associated with plasticity in circadian rhythms and G. Bloch will present data on possible interactions of JH with the circadian clock.

Although only at its beginnings, the diversity of hormonal effects in adult insects is evident and strikingly resembles comparable effects in vertebrates. Future research will have to show how JH acts at the cellular and biochemical level.

8 Juvenile Hormone Regulation of Reproductive Maturity and Sexual Signaling in Tephritid Fruit Flies

Peter Teal and Yeudiel Gomez-Simuta

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Tephritid fruit flies are quarantine pests of high economic importance through out the world. We have been studying the physiological mechanisms responsible for coordination of reproductive maturity and sex pheromone communication in males of several Caribbean Tephritid species. Mass spectroscopic analyses of extracts of hemolymph from sexually mature 12-day-old males resulted in identification of both juvenile hormone III and its bis-epoxide homolog in a ratio of 1:2.5. Analysis of extracts from mated and virgin 7-day-old males resulted in identification of three fold more juvenile hormone in extracts from mated males than in extracts from virgins. No juvenile hormone was found in extracts obtained from 1-day-old males. Application of juvenile hormone analogs to newly eclosed males accelerated both reproductive maturity and pheromone production by 4-5 days. We concluded that juvenile hormone levels increase with increasing age of adult male Tephritid Fruit flies that have a significant pre-reproductive period during the adult stage and that this hormone was pivotal in coordinating development of sexual signaling and reproductive maturity in these flies. We have used this information to develop hormone supplement therapy techniques using juvenile hormone analogs to dramatically improve mating efficiency by sterile males of a number of Tephritid fly species released to control outbreaks of these pests with the Sterile Insect Technique. This has resulted in a significant improvement in efficacy of Sterile Insect Technique Programs for controlling Tephritid Fruit fly pests.

9 Effect of Juvenile Hormone on Olfactory Guided Behaviour and on Central Nervous Processing of Odours in a Moth

Christophe Gadenne

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Male moths rely on female sex pheromones to find their mating partner and on plant volatiles for the detection of food sources.

In the adult male migrant moth, *Agrotis ipsilon*, sex pheromone responsiveness is age- and juvenile hormone (JH) dependent. Males exhibiting low JH level (young sexually immature or surgically JH-deprived males) do not respond to female-produced sex pheromones. On the contrary, males showing high JH level (fully sexually mature or JH-injected males) are attracted. JH is not acting at the peripheral level, as the antennal olfactory system is fully functional in newly emerged and JH-deprived males. By performing intracellular recordings of antennal lobe (AL) interneurons after stimulation of the antenna with sex pheromone, we found that the sensitivity of AL interneurons increases with age and JH level. Moreover, by manipulating the JH level of the male moth, we could either induce (in young males) or inhibit (in mature males) a high sensitivity of AL interneurons. The sensitivity and specificity of the AL interneurons invol-

ved in central plant volatile processing are age-independent. JH action seems therefore to be restricted to the maturation of the olfactory system in the macroglomerular complex, the sex pheromone specific part of the AL. These results reveal a specific developmental JH-dependent plasticity of central sex pheromone processing in the AL.

Another form of plasticity was revealed in the same moth species. We have shown that mating induces a fast transient inhibition of both behavioural and central nervous responses to sex pheromones. This plasticity is, however, JH-independent as the JH biosynthetic activity remained unchanged in newly mated males.

Juvenile Hormone III Influences Phonotactic Behavior by Female Crickets through Regulation of the Response Properties of Identified Auditory Interneurons

10

John F. Stout

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Female crickets (*Acheta domesticus*) recognize and respond phonotactically to the calling song (CS) of conspecific males. This behavior involves choosing an auditory stimulus that has the temporal structure (especially syllable period - SP) and carrier frequencies characteristic of the conspecific male's call, locating and walking to (phonotaxis) the source of the call. Phonotactic responsiveness usually begins 3 to 5 days following the imaginal molt as the result of decreasing behavioral thresholds and is accompanied by increased juvenile hormone III (JHIII) production. Removal of the corpora allata (the source of JHIII) causes short term increases of 20 dB or more in the phonotactic thresholds of females. Application of JHIII to allatectomized females whose phonotactic thresholds have increased causes rapid decreases in the behavioral threshold. The responses to model CSs of both the L1 auditory interneuron and the prolonged response of the L3 auditory interneuron become more vigorous with progressively lower thresholds during this period. The threshold for the prolonged response correlates with the decreasing behavioral thresholds, while L1's threshold is frequently 10 or more dB below the female's behavioral threshold. Application of JHIII to 1-day-old females causes reductions in their phonotactic thresholds, the response threshold for the L1 neuron and the prolonged response of the L3 neuron.

Virgin females, as they age, lose selectiveness in their phonotactic responses to SPs of the male's calls while maintaining maximal responsiveness. This is accompanied by reduced JHIII production and by reduced selectiveness of the immediate response by the L3 neuron. Experimentally raising the level of JHIII reverses this process, returning both selective phonotaxis and the selectiveness of the L3 neuron to a level typical of young females. This effect of JHIII on the SP-selective processing of the L3 neuron can be explained by hormonally induced changes in the inhibitory synaptic coupling of the ON1 auditory interneuron to the L3 neuron. Application of JHIII to females also causes increases in the expression of nicotinic receptor-like mRNA in the somata of both the L1 and L3 neurons.

Much of the development and plasticity of the female's phonotactic behavior is influenced by JHIII induced changes in the response properties of the L1, L3 and ON1 neurons. Since these neurons are not redundant and play necessary roles in this behavior, the

cricket system provides a powerful model for illuminating the hormonal regulation of the development and plasticity of behavior.

Supported by NSF IBN-9808834 and faculty grants from Andrews University.

11 Morphological and Functional Maturation in the Adult Locust Neuro-Muscular System Regulated by Juvenile Hormone

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Insect development and maturation is largely governed by hormones. The hormone playing a pivotal role during insect reproductive development is juvenile hormone (JH). This hormone has regulating effects on the development of gonads and accessory glands, but has also a strong influence on body muscles and the nervous system (Wyatt and Davey, 1996). Whether JH has the potential to alter biochemical and/or biophysical properties of muscle fibres or to adapt neuro-muscular performance to the requirements of stage specific behaviour, (e.g. reproductive behaviour) is largely unknown.

To address this question I studied an insect neuro-muscular system that undergoes a variety of structural and functional changes which enable the animal to perform adequate sex specific behaviour (oviposition).These characteristics make the system well suited for the investigation of JH influence on the neuromuscular system.

During oviposition, locusts extend their abdomen in a telescoping manner up to six times their normal length (superextension). The ability to superextend is enabled by various changes in the abdominal neuromuscular system. Changes consist of growth and maturation of muscle fibres in specific segments, stage dependent expression of ionic currents and alterations of their membrane properties. The growth of muscle fibres is paralleled by an increase in membrane capacitance and resting conductance. Experimental data revealed a 4-fold increase of the muscle fibre surface area whereas resting membrane conductance increased only 1.5-fold. During growth, muscles fibres elongate from 2.5 mm to 4 mm. Retrograde fills with biocytin showed that the axon collaterals of motoneurons innervating theses muscles do not sprout to cover the additional length of muscle. Instead, motoneuron terminals are restricted to a midline area and are absent from the posterior and anterior parts of mature muscles. In most invertebrate muscles depolarisation activated inward current is carried by calcium. In locust longitudinal muscles these calcium currents seem to change their activation threshold during reproductive development as revealed by two electrode voltage clamp experiments. Furthermore, the motoneurones supplying these muscles display altered activity which might serve to adapt the nervous system output to the structural and functional changes of the muscle fibres.

The described changes are largely dependent on juvenile hormone as suggested by experiments were the hormonal status of the animal was manipulated. These results suggest a remodelling of structural and functional properties in a neuro-muscular system to enable adequate reproductive behaviour. The information that is currently available

on the role of JH for reproductive development in insects suggest interesting similarities to the steroid regulated sexual maturation in vertebrates.

Supported by the Deutsche Forschungsgemeinschaft (RO-2122/2-1)

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Juvenile Hormone, Neurogenesis and Behaviour in the Adult Cricket

12

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In the female house cricket, *Acheta domesticus*, juvenile hormone (JH) is necessary to induce the expression of egg-laying behaviour. Once oviposition is "turned on", JH is no more required for its maintenance. To investigate the mechanisms by which JH was able to initiate this behaviour, we examined its actions on the young adult female brain. We discovered the persistence of dividing neuroblasts in the adult cricket mushroom bodies, and showed that their proliferation was stimulated by JH and inhibited by ecdysone. We also demonstrated that the stimulatory effect of JH on neuroblast proliferation was mediated by putrescine, a short chain polyamine. The dual effect of JH on triggering oviposition and enhancing neurogenesis led us to hypothesize that the newly generated neurones could play a role in the maturation of egg-laying behaviour. We thus developed a method to specifically kill mushroom body neuroblasts, using γ-irradiation of the adult cricket head. Irradiated crickets exhibited a 90% reduction of neuroblast proliferation, without altering mature neuron survival or ovarian development. However, quantification of oviposition behaviour by radiotracking failed to evidence any difference between control and irradiated females. We can thus conclude that adult neurogenesis is not involved in the development of egg-laying behaviour induced by JH. By contrast, irradiated crickets are deficient in an operant olfactory conditioning, suggesting the participation of the newborn neurons in learning and memory processes.

Juvenile hormone and task-related plasticity in circadian rhythms in the honey bee

13

Guy Bloch

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Juvenile hormone (JH) influences age-related division of labor in honey bees. Young worker bees labor inside the nest with low JH hemolymph titers and biosynthesis rates. Older bees (> 3 weeks of age) typically forage for nectar and pollen and have high JH hemolymph titers and biosynthesis rates. Treating young bees with JH, JH analogs, or JH mimics cause precocious onset of foraging while allatectomized bees with no circu-

lating JH start to forage later than shame operated or intact bees. Division of labor is also associated with plasticity in circadian rhythms. Young nurse bees attend the brood around the clock with no circadian rhythms whereas foragers have robust rhythms that are used for sun compass navigation and timing visits to flowers. Worker maturation is also associated with a developmental increase in brain mRNA and protein levels of the clock gene period (per). Based on these observations we tested the hypothesis that the circadian system and JH interact. Treatments with the JH analog methoprene or allatectomy did not influence the onset of rhythmicity, overall locomotor activity, or the free-running period of rhythmic locomotor behavior. These suggest that JH does not coordinate all aspects of division of labor in bees and that coordination of task performance with circadian rhythms is probably mediated by other regulatory systems. Measurements of JH titers in foragers collected throughout the day suggest that JH levels oscillate with significant diurnal rhythms and high levels during the night. Taken together our findings are consistent with the premise that the circadian clock influences JH titers but we find no evidence for JH influences the clock.

14 Juvenile Hormone Binding Proteins and Neuronal Plasticity

Yohann Gaubard [1], Christophe Gadenne [2], Glenn Downe Prestwich [3], Christer Löfstedt [1] and Jean-Francois Picimbon [1]

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Juvenile hormone (JH) is traditionally regarded as the main regulatory factor of the developmental processes in insects. More recently, JH has been pointed out as a major hormone regulating reproductive development in adult insects. Besides its influence on the maturation of reproductive organs, JH also regulates sexual and other age-related behaviours by influencing the morphology and function of both nervous and muscular systems. In particular, JH has been found to control pheromone production and responsiveness to sex pheromone in the Lepidopteran species *Agrotis ipsilon* (Gadenne et al. , 1993; Picimbon et al. , 1995). In this species, it has been shown that JH may be involved in the central nervous processing of sex pheromone by controlling the sensitivity of antennal lobe (AL) interneurons to sex pheromone (Anton and Gadenne, 1999). The action of JH seems to be restricted only to the central processing of sex pheromones ; the central processing of plant odours have been shown to be independent of JH (Greiner et al. , 2002). This suggests that JH may act in the macroglomerular complex, and in particular in the AL region processing sex pheromones, by controlling neuronal plasticity in the moth brain. To elucidate the molecular mechanism underlying JH regulation, we addressed the existence of Juvenile Hormone Binding Proteins (JHBPs) in the brain of moths by photolabeling experiments. The tritiated JH I analog, epoxybishomofarnesyl diazoacetate ([3H]-EBDA), covalently bound to specific proteins in both cytosolic and cytoplasmic membrane protein extracts from brains and fat bodies from A. ipsilon adults. In particular, [3H]-EBDA bound to a protein with a molecular weight of 32 kDa in the cytosolic fraction of brain-suboesophageal ganglion extracts. A 35 kDa-protein was detected in the membrane fraction of Br-SOG extracts. Similar results were obtained using fat bodies that are considered as main target tissues for JH. We therefore

speculate that various JH-regulated tissues may express very similar JHBPs and that JH could act through two different intracellular pathways (Picimbon, 1995). Planned projects address the molecular characterization, the *in situ* localization and the functional expression of JHBPs in the insect brain.

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Research Project nº1065-02 (INRA-Santé des Plantes et Environnement)

Effects of juvenile hormone on the abdominal motor system of adult *Locusta migratoria*

15

Roland Spieß and Uwe Rose

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Insect development is governed by two major hormones: ecdysteroids and juvenile hormone (JH). Unlike its name implies, juvenile hormone has multiple effects in the adult insect, particularly on tissue involved in reproductive development (Wyatt 97; Wyatt and Davey 96). Rose et al (2001) showed morphological (growth) and functional modifications of longitudinal muscles being restricted to oviposition segments of *Locusta migratoria*. These modifications depend on the presence of JH during maturation. To figure out if there are corresponding effects on neuronal level, we investigated the impact of JH on the excitability of motoneurons in the abdominal system of *Locusta migratoria*. The experiments were performed on an isolated nerve cord by recording the activity of a peripheral nerve innervating a longitudinal abdominal muscle in segment 6 following a stimulation of the connective. The influence of JH on the excitability of motoneurons in the abdominal system was investigated by modifying the hormonal status of female Locusts that included surgical allatectomy, application of the JH analogue methoprene and transplantation of active corpora allata (CA), the gland producing JH.

The results show that JH has an impact on the excitability of the abdominal system: mature females show a much higher and prolonged nerve activity following a stimulation than do immature ones. Allatectomy prevents the system from becoming more sensitive to stimulation. We could also demonstrate that the effect of a higher excitability is restricted to those segments involved in oviposition. This is consistent with the findings of Rose et al (2001) who showed modifications of muscle properties in oviposition segments only. Thus enhanced excitability of motoneurons may ensure appropriate input to the growing muscle. Neither transplantation of a pair of CA to immature females nor application of the JH analogue methoprene triggered a precocious increase of excitability. Surprisingly also the application of methoprene to allatectomised females did not re-establish the elevated excitability of the system to the level of untreated mature animals within 14d. We conclude that though methoprene is capable of mimicking JH on the level of morphology (the treated animals showed development of reproductive organs and eggs) it does not mediate a recognisable effect on the nervous system.

Currently we are using the natural occurring juvenile hormone III instead of an analogue to test for differences.

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16 Olfactory-guided aggregation behaviour and olfactory processing in desert locusts are regulated by juvenile hormone

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Adult gregarious desert locusts, *Schistocerca gregaria*, aggregate in large swarms and cause huge damage on a variety of host plants in large parts of Africa and Asia. Aggregation behaviour is elicited by an odour blend produced by mature male locusts, which is attractive for both adult males and females. The attractiveness of the major active compound, phenylacetonitril, changes, however, during adult life. In olfactometer studies, we could show that adult desert locusts are only reacting to the aggregation pheromones during the first two weeks of their adult life and are later indifferent to the odour. These behavioural changes are in correlation with an increasing juvenile hormone (JH) level with increasing age. In ablation experiments, locusts without corpora allata, the glands where JH biosynthesis takes place, continued to be attracted by the aggregation pheromone throughout adult life. On the other hand, young adult locusts injected with JH were indifferent to the aggregation pheromone.

To investigate the neuronal basis of the JH-mediated behavioural changes, we first performed electroantennograms (EAG) on locusts of different age and JH-manipulated individuals during stimulation with aggregation pheromone. No differences in EAG responses were found between the different groups of locusts. We conclude that peripheral receptor neurons do not change their response characteristics with age and changing JH level.

Intracellular recordings performed on a large number of antennal lobe projection neurons showed that the proportion of neurons responding to aggregation pheromones changed significantly with age and depending on the JH level. Young locusts and JH-deprived old locusts possess a larger proportion of projection neurons sensitive to aggregation pheromones than old locust or young JH-injected individuals. The effect of age and JH on the level of the primary olfactory centre, the antennal lobe, might play an important role on the behavioural changes observed.

Introductory Remarks to Symposium 3

Cytokines as mediators of neuroglial interactions

Jörg Mey and Heike Siebert

After traumatic nerve injury, in ischemic brain damage and in neurodegenerative diseases, it is of foremost clinical concern to prevent nerve cell death and to develop strategies for the support of axonal regeneration. This requires an understanding of traumatic processes in the nervous system and of their regulation by intercellular signals. Originally deriving from immunological research the cytokine concept has gained increasing relevance in this context. In the CNS, cytokines mediate interactions between astrocytes, microglia cells, neurons and, under pathological conditions, infiltrating leukocytes from the circulation. Peripheral nerve lesions also activate paracrine signals between macrophages, Schwann cells and neurons. Cytokines are polypeptides that bind with high affinity to specific cell surface receptors and activate intracellular second messenger cascades. Unlike hormones, cytokines are not stored in glands as preformed molecules but are rapidly synthesized and secreted by a variety of cell types after stimulation. In development and under pathological conditions they act on many different targets and frequently affect the action of other cytokines in a synergistic or antagonistic manner. Their physiological functions in the nervous system will be discussed in this symposium.

Stefan Wiese's contribution focuses on the neuropoietic cytokines (including IL-6, CNTF, LIF). They share the gp130-family of receptors that activate janus kinases and the STAT transcription factors. This pathway is activated as part of the immediate inflammatory reaction. In addition, neurotrophic properties of CNTF and LIF have been reported for various neuronal populations. Discussed by Hans Werner Müller, the chemokines comprise a large family of small proteins, who mediate their biological effects through G-protein-coupled receptors. They are primarily characterized as chemoattractants of hematogenous cells. Chemokines and matrix metalloproteinases are the subject of Heike Siebert's talk. Various metalloproteinases appear in the nervous system, in particular after blood brain barrier leakage, and contribute to the removal of extracellular matrix. Gennadij Raivich has investigated a number of cytokines including TGFβ and TNF. Binding of TGFβ to cell surface receptors requires its local release from a latency associated peptide. In consequence, Smad-proteins are phosphorylated in the target cell and translocate to the nucleus, where they form heteromeric complexes to regulate gene transcription. Astrocytes, oligodendrocytes, microglia and neurons have been shown to be targets of TGFβs which tend to cause cell cycle arrest and differentiation. TGFβ activates ECM deposition by astrocytes and fibroblasts and also modulates the activity of a large number of other cytokines that are involved in immune reactions. TNFα effects are also mediated by plasmamembrane receptors. Via recruitment of intracellular adaptors proteins it can trigger apoptosis or activate the transcription factors NFκB and JUN. Its functions in peripheral nerve de- and regeneration will be covered by Claudia Sommer. In contrast to the cytokines proper, the lipophilic retinoic acid penetrates cellular membranes. Its receptors are localized in the cell nucleus where they act as ligand-activated transcription factors. Jörg Mey will discuss the role of retinoic acid as a regulator of cytokines after peripheral nerve injury.

17 Neuroprotective effects of neurotrophic factors: Basics and clinical application

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In higher vertebrates, motoneurons are generated in excess during embryonic development, and a significant proportion of the newly generated cells die after they have made functional contact with their target. Thus, embryonic motoneurons serve as a model for studying processes underlying the pathological changes in various forms of human motoneuron disease. Motoneurons are maintained by neurotrophic factors which are produced by skeletal muscle and glial cells. So far, a variety of these factors have been identified. They support motoneuron survival in cell culture, and gene inactivation by homologous recombination has shown that these factors play together in supporting survival of these cells, both during embryonic and postnatal development. Investigations with isolated motor and sensory neurons have shown that neurotrophic factors promote a more than 25-fold upregulation of members of the IAP/ITA-family. The avian ITA is homologous to the baculoviral and mammalian inhibitor of apoptosis (IAP) proteins, which prevent apoptosis by inhibition of specific caspases. Overexpression of ITA in primary neurons support survival of these cells in the absence of neurotrophic factors, and ita antisense constructs inhibit neurotrophic factor-mediated survival. These data indicate that upregulation of members of the IAP/ITA-family is an essential signaling event for survival of neurons, and IAPs might be important target genes for neurotrophic factors both under physiological and pharmacological conditions.

In order to investigate the signaling pathways which lead to upregulation of members of the IAP/ITA family, we have focussed on the role of Raf-kinases. These serin/threonin-kinases play an essential role in coordinating various signaling pathways, including the MAPK pathway, the ΠЗK/Akt pathway and others. The Raf-kinase family in mammals includes 3 members, A-Raf, B-Raf and C-Raf. Mice in which B-Raf or C-Raf is deleted die during embryonic development between day 12 and 19 due to various organ defects. Therefore, we have isolated motoneurons from E12 C-Raf- and B-Raf-deficient mice and investigated their capacity to respond to neurotrophic factors for survival. Mice lacking C-Raf could survive in the presence of neurotrophic factor indistinguishable from wildtype. In contrast, B-Raf-deficient mice lost their response to neurotrophic factors. This appears interesting, as the B-Raf-kinase, in contrast to C-Raf, is activated by extracellular signals which lead to elevated cAMP. Thus, B-Raf could be an important integrator of neurotrophic factors and neural activity through neurotransmitter receptors.

Previous studies in which neurotrophic factors such as CNTF, BDNF, or IGF-I were systemically applied to patients with amyotrophic lateral sclerosis (ALS) did not show significant effects on the progression of the disease. This might be due to the fact that neurotrophic factors, at least under physiological conditions, act at sites of direct cellular contact and normally do not reach their target cells by transport through the bloodstream. Therefore, the pharmacokinetic conditions provided by systemic administration might not be optimal for treatment of neurodegenerative disorders. Animal studies have demonstrated that at least one of these neurotrophic factors, BDNF, can be taken up by

motoneurons after continuous pump administration into the subarachnoidal space. Nethertheless, these first clinical studies did not show efficacy in multicenter Phase 3 clinical trials. These data indicate that new strategies which can increase the local supply of neurotrophic factors to specific populations of neurons or techniques to stimulate downstream signaling pathways production that promote survival and maintenance of neurites might be necessary for new therapeutic approaches to neurodegenerative disorders.

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SDF-1 chemokines in the mammalian nervous system: Expression, regulation and function

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The cytokines SDF(stromal derived factor)-1 α and SDF-1 β are two alternatively spliced variants of the CXC subfamily of chemokines that are highly conserved among species. SDF-1 chemokines and their CXCR-4 (LESTR/FUSIN)receptor serve wide spread functions in haematopoiesis, HIV pathogenesis, host defense, vascular development and neuronal patterning. We have recently cloned SDF-1 γ, a new SDF-1 isoform (Gleichmann et al., 2000, Eur. J. Neurosci. 12, 1857-1866). SDF- 1 γ comprises 119 amino acids including a unique C-terminal 30 amino acid sequence that contains several motifs for proteolytic cleavage giving rise to putative novel neuropeptides. We report on the cellular (neuronal and glial) expression and regional distribution of SDF-1 isoforms and CXCR-4 during development and maturation of the PNS and CNS. SDF-1 β and –1 γ mRNA are inversely regulated in Schwann cells during peripheral nerve development. While SDF- 1 β is the predominant isoform in embryonic and early postnatal nerve, SDF- 1 γ mRNA is expressed at elevated levels in the adult PNS.

SDF-1 α, however, could, thus far, not be detected in the nervous system. The regulation of CXCR-4 receptor expression was investigated using quantitative RT-PCR and the interaction of SDF- 1 γ with Schwann cells, cerebral astrocytes and neocortical neurons during development *in vitro* as well as CXCR-4 receptor activation could be monitored by calcium imaging. Interaction of SDF- 1 γ with Schwann cells was shown to induce CXCR-4 mediated apoptosis. In addition, we will report on the neuromodulatory effect of SDF-1 chemokines and novel peptides derived from the C-terminal sequence of SDF-1 γ on the spontaneous spiking pattern recorded in primary neocortical cultures grown on multielectrode arrays (NeuroChip).

19 Cytokines and proteases that influence sciatic nerve degeneration

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In the central and peripheral nervous system the loss of myelin and axonal dysfunction is of certain interest in particular in terms of degenerative and inflammatory diseases like multiple sclerosis. The destruction of myelin and the invasion of peripheral blood cells has an evident meaning for inflammatory events and can be examined in animal models for Wallerian degeneration. Here we used the model of sciatic nerve axotomy in order to examine the effects of different chemokines, cytokines, adhesion molecules and matrix metallopoteinases (MMPs) on macrophage invasion during peripheral nerve degeneration. The investigations have been conducted by means of expression studies on chemokines and MMPs and with knockout mice for these proteins.

For the chemokine receptors we found an essential role of CCR2 during macrophage invasion into the degenerating distal nerve stump. Also the mRNAs of CCR2 ligands (JE/MCP-1 and MCP-3) are upregulated during the early phase of nerve degeneration as well as MMP-9, which is peaking at two different timepoints. In knockout mice for the cell adhesion molecule ICAM-1 and for the cytokine TNF-α we found reduced numbers of macrophages, higher amounts of preserved myelin and extended numbers of preserved axons. In contrast, mice deficient for interleukin-6 or inducible nitric oxide synthetase (iNOS) or even MMP-9 knockouts did not show any significant macrophage reduction.

In conclusion, our data suggest an important role for cell adhesion and cell attraction by chemokines on macrophages during the early phase of Wallerian degeneration after sciatic nerve axotomy. Furthermore the secretory products of these cells namely MMPs and TNF seem to influence the ongoing time course of degeneration.

20 Cytotoxic Potential of Inflammation-associated Cytokines in Neuronal Degeneration: Role of TNF-α and TGF-β1

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Neural injury triggers inflammatory changes in neighboring glia (astrocytes, microglia), recruitment of leukocytes and synthesis of the cytokines MCSF, IL1-β, IL6, TNF-α, TGF-β1 and IFN-γ. These cytokines could assist in neural repair but also cause secondary neuronal damage. We examined the effects of transgenic deletion of these cytokines and receptors on neuronal survival and non-neuronal response in the facial motor nucleus after nerve cut.

Axotomy leads to a rapid induction of mRNA for MCSF receptor, TGF-β 1 and IL6 (peak: day 1-4) and a later increase for IL1-β, TNF-α and IFN-γ, coinciding with a peak

of neuronal cell death and microglial phagocytosis at day 14 (d14). Deletion of MCSF or IL6 interfered with early lymphocyte recruitment and microglial response (d1-4), but did not affect neuronal cell death (d30). Deletion of IFN-γ receptor 1 (IFN-γ R1) had no effect. Deletion of IL1R1 or TNFR2 inhibited late lymphocyte recruitment (d14); deletion of TNFR1 inhibited microglia surrounding phagocytic microglial nodules but did not affect neuronal survival. Only combined deletion of TNFR1 and TNFR2 caused a striking absence of phagocytotic microglial nodules (d14-30), and almost complete prevention of cell loss by d30.

A similar effect, of reduced neuronal cell death after facial axotomy was also observed in TGF-β1-deficient mice. *In situ* hybridisation and immunohistochemistry showed prominent upregulation of TGF-β1 on activated microglia, and TGF-β receptors 1 and 2 on injured motoneurons, pointing to microglial secretion of cytotoxic molecules acting on sensitised injured neurons.

In summary, injury-associated cytokines play a key role in inducing different aspects of neural response including neuronal cell death. Here, cell death depends on a combination of several pathways mediated by TNF and TGF-β receptors.

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Expression and transport of tumor necrosis factor-α in peripheral nerve injury

21

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Tumor necrosis factor-α (TNF) is rapidly upregulated in injured peripheral nerves at the mRNA and protein level with a peak around 12 hours after an injury. In the injured peripheral nerve itself, TNF is predominantly produced in Schwann cells. TNF receptors, in particular TNF-R 2, are concomitantly upregulated with a more prolonged time course. In dorsal root ganglia (DRG), the spectrum of neuronal profiles displaying TNF immunoreactivity shifts from the small to the medium sized neurons after nerve injury. In these neurons, TNF is colocalized with neurofilament and trkB. Furthermore, in an incomplete nerve injury, this shift is not only obvious in the injured population, but also in the adjacent uninjured population of neurons. TNF protein, either produced in neurons or in Schwann cells, is transported along the nerve in an anterograde direction, and radioactively labeled TNF can be detected in muscle after intraneural injection. These

findings suggest a role of TNF in peripheral nerve de- and regeneration and possibly in sensory disturbances associated with nerve injury.

22 Retinoic acid as a regulator of cytokine signaling in peripheral nerve regeneration

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In mammalian peripheral nerves a crush lesion causes interactions between injured neurons, Schwann cells and hematogenous macrophages that can lead to successful axonal regeneration. We suggest that the transcriptional activator retinoic acid (RA) takes part in gene regulation after peripheral nerve injury. This hypothesis is supported by the following results: (1) While all necessary components of the RA signaling pathway were detected in the sciatic nerve of adult rats, we found local activation of RA responsive elements only after sciatic nerve injury. Nerve crush or transection resulted in a significant upregulation of CRBP-I, which is thought to facilitate the synthesis of RA and of CRABP-II, a possible mediator of RA transfer to its nuclear receptors. Upregulation of CRBP-I and CRABP-II provide putative triggers of RA activation in the PNS. (2) In various cell culture systems, including Schwann cell primary cultures, retinoids were found to interact with cytokine signals that mediate cellular interactions after nerve lesions *in vivo*. (3) Previously published data demonstrate that endogenous RA promotes glial and neuronal differentiation during development, including the outgrowth of axons in the developing spinal cord, cerebellum, dorsal root ganglia and sympathetic ganglia. Axonal regeneration of differentiated retinal ganglion cells and peripheral sensory neurons is enhanced by RA *in vitro*.

23 Activation of retinoic acid signaling after sciatic nerve injury: Upregulation of cellular retinoid binding proteins

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Recent experiments implicated the transcriptional activator retinoic acid (RA) as a regulator in neuroglial interactions after peripheral nerve injury (Mey et al., Soc. Neurosci. Abstr. 2002: 529.13). Based on the present results we suggest that RA signaling is activated via the cellular retinoic acid binding protein (CRABP)-II. With RT-PCR and Western blotting all necessary components of the RA signaling pathway were detected in the sciatic nerve of adult rats. These are retinoic acid receptors, retinoid X receptors, the retinoic acid synthesising enzymes RALDH-1, -2, and -3, the retinoic acid catabolizing enzyme CYP26, in addition, the cellular retinol binding protein CRBP-I and the cellular retinoic acid binding proteins CRABP-I and -II. Enzyme activity of RALDH-2 but not of RALDH-1 or -3 was detectable in the nerve. In a transgenic reporter mouse we found local activation of RA responsive elements in the regenerating sciatic nerve, while no RA signalling was observed in the non-injured peripheral nervous system. The crush nerve injury resulted in a more than 10-fold significant upregulation of CRBP-I, which is thought to facilitate the synthesis of RA, and of CRABP-II, a possible mediator of RA transfer to its nuclear receptors. Compared to the crush, a nerve transection

caused an even higher expression of CRABP-II, while the expression of CRBP-I was similar in both types of injury. Transcript expression and protein concentration of CRBP-I and CRABP-II were downregulated again 14d after nerve crush but remained high after transection when axonal regeneration was prevented. In contrast to the retinoid binding proteins, RALDH-2 immunoreactivity showed no significant increase after nerve injury. Our present data, showing strong and significant upregulation of CRBP-I and CRABP-II after sciatic nerve lesions in connection with the activation of an RAREreporter gene *in vivo*, argue for a novel function of retinoid signalling in the adult PNS.

Cytokine Expression in Schwann Cell Primary Cultures after Retinoic Acid Treatment

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Cytokines mediate important neuroglial interactions in the peripheral and central nervous system. In the PNS, Schwann cells appear to be the main source of some of these trophic factors. While a peripheral nerve injury causes differential regulation of many cytokines and neurotrophins, their molecular regulators are not known in many cases. In cell culture systems unrelated to the nervous system the transcriptional activator retinoic acid was found to interact with most cytokine signals that mediate cellular interactions after nerve injury. We are therefore investigating the retinoic acid-mediated transcriptional regulation of cytokines in Schwann cell primary cultures.

Schwann cells taken from sciatic nerves of newborn rats were treated with 100, 10, 1 nM all-trans RA and DMSO as control. After time intervals of 24, 48 and 96 hrs RNA was extracted. Transcript concentrations of various rat cytokine and neurotrophin genes were assessed by the RNase protection assay (RPA), the Affymetrix gene chip array or quantitative RT-PCR (Roche Light Cycler). Supernatants of Schwann cell primary cultures were analyzed using ELISA with antibodies against TGFbeta1, TNFalpha, IL-2, and IL-12.

The following cytokines and neurotrophins were expressed in Schwann cell primary cultures: NGF, BDNF (RPA, gene chip), GDNF (RPA), CNTF (RPA, RT-PCR), LIF, IL-6 (RT-PCR), TGFbeta1 (RPA, gene chip), TGFbeta2, TGFbeta3, IFNbeta, IFNgamma, MIF (RPA). Cell culture supernatants contained 80-100 pg/ml TGFbeta1 but, in contrast to the gene expression data, no IL-6. Proteins of IL-2, IL-12 and TNFalpha were detected but not significantly higher than in control medium.

The following cytokine transcripts were not detected in Schwann cells: TNFalpha, TNFbeta, GM-CSF and Lymphotoxin-β. Signals of TGFbeta2 and TGFbeta3, although seen with RPA, were very low in the gene chip expression assay.

Although in various other cell culture systems retinoic acid has been reported to regulate all of these cytokines, we did not see any RA-dependent differential regulation in Schwann cells with the RPA. As measured with quantitative RT-PCR, the expression of CNTF decreased after RA-treatment. Transcript concentration declined to 63% ($n = 6$, SEM = 4 %, $p < 0.0005$).

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25 The influence of different cytokines and proteases on sciatic nerve degeneration - a study in different knockout mice

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The loss of myelin and axonal dysfunction has become of outstanding interest with respect to degenerative and inflammatory diseases of the central and peripheral nervous system. In particular in terms of demyelinating diseases such as multiple sclerosis it is important to know the mechanisms which are responsible for the degeneration and destruction of myelin.

We examined the distally transected nerve segments of different knockout mice lacking the genes for chemokine receptors (CCR2 and CCR5) or cytokines (TNF-α; iNOS; IL-6) or matrix metalloproteinase 9 (MMP-9) or the cell adhesion molecule ICAM-1 six days after axotomy. Despite a distinct number of invading macrophages which phagocytosed most of the myelin and axonal debris, we were able to demonstrate, that animals which are deficient for the chemokine receptor CCR2 show a significantly lower number of invading macrophages and higher amounts of preserved myelin. Mice deficient for ICAM-1 or TNF-α also showed lower numbers of macrophages within the degenerating nerve. In contrast, knockout mice for iNOS, IL-6 and MMP-9 demonstrated no significant reduction of macrophage numbers.

Since macrophage invasion is known to be impaired in the absence of CCR2 as well as ICAM-1 and TNF-α, these data indicate an essential role of these cells and their secreted factors, namely TNF-a, but not nitric oxide or IL-6 in the destruction of the peripheral nervous system.

26 Quinolinic acid lesions of the caudate putamen in the rat lead to an increase of Ciliary Neurotrophic Factor

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In various animal models of Huntington's disease CNTF has previously been shown to be a potent neurotrophic factor when applied prior to excitotoxic lesions. Until now, data concerning the endogenous CNTF-expression after a quinolinic acid lesion in the caudate putamen (CPu) of the adult rat were rare. Using immunohistochemistry, we previously observed an increase of protoplasmatic astrocytes which contained this protein one month after a quinolinic acid lesion. Therefore, we now quantified the increase of CNTF-protein in the CPu using Western blotting. Six male rats received two unilateral injections of quinolinic acid into the CPu (2 x 0.5 µl/0.09 M/pH 7.4; stereotaxic coordinates according to bregma: AP +1.2/±0; ML +2.8/+3.6; V -5.5/-5.5) and furthermore

two sham injections consisting of DMEM into the contralateral CPu (respective coordinates). Successful lesions were evaluated by apomorphine-induced rotations (1 mg/kg body weight over 30 minutes) 28 days after lesion. Mean apomorphine-induced rotations were about 4.9 (±1.1) per minute. Four animals were then perfused with 4°C isotonic saline and tissue blocks of the lesioned and the contralateral sham-lesioned CPu were boiled in SDS-lysis buffer. Both CPu of two intact animals were also prepared and served as controls. The brains of the remaining two lesioned animals were cut in a cryostat and brainslices through the CPu were stained for myelin or with cresyl violet. Lysates, consisting of about 1.5 mg fresh-tissue weight, were loaded per lane of a 4-20% SDS-PAGE-Gel and then blotted. Membranes were incubated with primary antibodies against CNTF (derived from goat, 1:1000, R&D Systems) and the housekeeping protein ß-actin (mouse monoclonal, 1:3000, Sigma) and finally detected with an ECL-Kit on x-ray films, which were used to quantify the increase of CNTF after lesion. By detecting various concentrations (0.05-0.5 ng) of recombinant rat-CNTF (R&D Systems) the specificity of the used antibodies was shown and the CNTF-content in the different CPu could be defined. The x-ray films were digitized by means of a high resolution flat-bed-scanner at 800ppi (256 gray levels) and corrected for background fluctuations. CNTF dots were delineated by an image analytical method (KS400, Zeiss Vision) and their absolute expression calculated by using the determined exponential distribution of known CNTF-standards. A significant ($p<0.01$, t-test) increase of CNTF (0.2 ng/1500 µg fresh weight (FW)) in the quinolinic-acid lesioned CPu was observed, compared to the sham-lesioned (0.08ng/1500µg FW) and the intact control CPu (0.07ng/1500µg FW). Further studies evaluating the expression of other neurotrophins in this lesion model, for example LIF (leukemia inhibitory factor) or GDNF (glial cell line derived neurotrophic factor), are under investigation because they may be involved as well in mediating lesion-induced activation of astrocytes.

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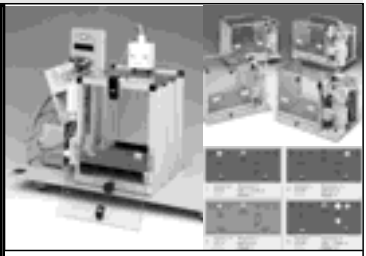

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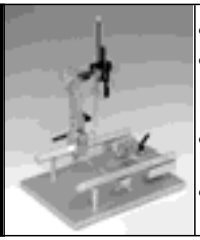

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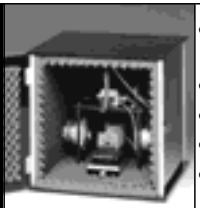

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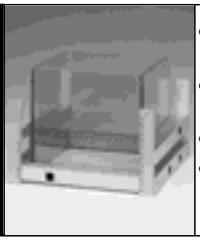

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Introductory Remarks to Symposium 4

Transgenic animal models of neurodegenerative diseases

Jörg B. Schulz and Christian Haass

Identification of genetic causes underlying either typical hereditary neurodegenerative diseases (e.g. Friedreich's ataxia) or rare hereditary forms of typically idiopathic neurodegenerative diseases (e.g. Alzheimer's and Parkinson's disease) has raised new opportunities to study the pathogenesis of these neurodegenerative disorders. Although molecular and biochemical consequences of mutations may be studied in cell lines and primary cell cultures, only animal models allow to study functional consequences in a complete organism, in their biological context and the consequences in behavior. Model systems like *C. elegans* and *D. melanogaster* allow to study organisms from birth to death in a short time period. Furthermore, hypotheses can be tested rapidly by simple and quick genetic manipulations. In the year 2002 the nobel prize committee honored researchers who identified *C. elegans* as a model system for disease and who helped to identify its complete genome.

Philipp Kahle will discuss transgenic mouse models of synucleinopathies based on ectopic expression of disease-related α-synuclein. Somatodendritic accumulation of α-synuclein was observed in dopaminergic neurites. Ultimately, formation of Parkinson's disease pathology causes severe locomotor dysfunction in transgenic mice. Oligodendroglial expression of α-synuclein induces pathological and biochemical changes resembling multiple system atrophy.

Hélène Puccio will review her work on frataxin-deficient mice. Whereas knockout mice are intrauterine lethal, mice with a neuronal or muscular deficiency of frataxin develop behavioral symptoms, pathological and biochemical changes soon after birth and have a life expectancy of only 5 and 9 weeks, respectively. They serve as valuable animal models for Friedreich's ataxia.

Bart de Strooper will focus on the physiological function of presenilins and pathological changes occurring in Alzheimer's disease-associated mutations using transgenic mice. Similarly, Frank Heppner will review transgenic mice as models for prion disorders.

Finally, Ralph Baumeister will discuss *C. elegans* as a model system for Parkinson's and Alzheimer's disease, allowing to study the consequences of disease-associated mutations in α-synuclein, parkin, presenilins and amyloid.

27 Transgenic Mouse Models of α-Synucleinopathies

Philipp Kahle

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Fibrillar aggregates of the presynaptic protein α-synuclein form Lewy bodies and Lewy neurites that are diagnostic for Parkinson's disease and pathologically related diseases (dementia with Lewy bodies, neurodegeneration with brain iron accumulation type 1 (formerly known as Hallervorden-Spatz disease), and pure autonomic failure). Moreover, (oligodendro)glial cytoplasmic inclusions composed of α-synuclein characterize multiple system atrophy. In order to generate transgenic mouse models for these diseases collectively referred to as α-synucleinopathies, we have expressed wild-type and the Parkinson's disease associated A30P mutant α-synuclein throughout the central nervous system neurons (Thy1 promoter), selectively in catecholaminergic neurons (tyrosine hydroxylase promoter), and in oligodendrocytes (proteolipid protein promoter). Abnormal accumulation of insoluble transgenic α-synuclein in neuronal cell bodies and swollen neurites occurred in young mice. This early pathology aggravated to *bona fide* Lewy pathology in a time and gene dose dependent manner. In >1.5 year old heterozygous and >0.5 year old homozygous (Thy1)-h[A30P]α-synuclein mice, we detected misfolded α-synuclein *in situ* based on the proteinase K-resistance of α-synuclein fibrils. Moreover, diagnostic hyperphosphorylation at serine-129 as well as oxidiation of α-synuclein were found using specific antibodies, concomitant with formation of argyrophilic, thioflavin-positive and electron-dense inclusions that were occasionally ubiquitinated. α-Synuclein pathology in the transgenic mice was predominantly in the brain stem and spinal cord. Astrogliosis was found in these heavily affected tissues. The transgenic mice showed a progressive deterioration of locomotor function, ultimately leading to premature death. Transgenic α-synuclein expressed in oligodendrocytes also accumulated in an insoluble, pathologically phosphorylated form. Thus, misfolding and (hyper)phosphorylation of α-synuclein may cause dysfunction of affected brain regions, as reflected in transgenic mouse models of human α-synucleinopathies.

28 Mouse models of Friedreich's ataxia- models for oxidative stress

Hélène Puccio

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Friedreich ataxia, the most common form of inherited ataxia, is a decade long disabling disease that associates peripheral neuropathy, spinal cord degeneration and cardiomyopathy. Friedreich ataxia is caused by partial deficiency of frataxin, a mitochondrial protein most likely involved in iron-sulfur protein biogenesis and/or iron homeostasis, due to an intronic expansion in the defective gene. We recently have generated two distinct conditional knockout lines: a striated muscles restricted knockout to decipher the cardiac symptoms associated with Friedreich's ataxia and a neuron-restricted knockout to elucidate the neurological symptoms. Both conditional knockout lines reproduce

important pathophysiological and biochemical features associated with Friedreich's ataxia: i) progressive cardiac hypertrophy, ii) progressive ataxia and loss of proprioception, iii) multiple Fe-S dependent enzyme deficiency in affected tissues, iv) time-dependent intramitochondrial iron accumulation. We have used these mouse models for therapeutic testing of different antioxidant compounds.

In order to develop a mouse model that recreates only the neurological features of the human disease, we have used the tamoxifen inducible system under the control of a neuron-specific prion promoter in order to have a spatio-temporally controlled deletion of the mouse frataxin gene. We have obtained two different lines which exhibit a progressive neurological phenotype with slow evolution. Accelerating rotarod measurements revealed a general locomotor deficit beginning at around 10 weeks. We have further analyzed this locomotion defect by footprint analysis which clearly demonstrates that the mutant animals present a progressive ataxia until loss of spontaneous ambulation at around 1 year of age. EMG studies show normal sensory nerve conduction on the caudal nerve and normal motor evoked potential. In contrast, there is a significant decrease in the sensorimotor reflexes after sciatic nerve stimulation ($p<0.01$) indicating that the large myelinated proprioceptive sensory neurons are functionally defective causing sensory and spinocerebellar ataxia, a distinctive pathological characteristic of FRDA. Preliminary histological data show both spinal cord and dorsal root ganglia pathology, with no sensory axonal defect. These mutant mice represent therefore an excellent model of the human disease and will be tested with anti-oxidant compounds, currently the best pharmacological candidates against the partial frataxin deficiency and ensuing mitochondrial defects.

Integral membrane proteolysis mediated by the presenilin/γ-secretase complex

29

Bart de Strooper

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The presenilins (PS) were originally discovered by geneticists searching for mutations causing early onset familial Alzheimer's Disease. This discovery was followed rapidly by a real explosion of publications, first linking PS to Notch signalling in *C. elegans* and later to a variety of other signalling processes. Several lines of evidence imply PS also in apoptotic events and in Ca^{2+} signalling. They are involved in the regulated intramembrane proteolysis of several receptors explaining partially the many physiological processes in which PS are involved. These roles are conserved throughout evolution since PS are even found in plants. The central role of PS in many biological processes raises the question whether they are good drug targets for Alzheimer's disease. Some evidence however indicates that there are still some opportunities to modulate Abeta peptide production via PS/γ-secretase modulation in a possible therapeutical relevant way. One of the aims is to precisely delineate how presenilin associated proteins like nicastrin, aph1a&b and Pen2 modulate γ-secretase function and how different complexes could have specific roles in physiological relevant processes. We are at the beginning of a highly interesting expedition and the combination of genetic, cell biological and biochemical investigations in worms, flies and mice allows us to paint a (partial) picture of the γ-secretase complex and its functions in health and disease.

30 Transgenic mouse models of prion disorders

Frank L. Heppner and Adriano Aguzzi

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Prion diseases are inevitably fatal neurodegenerative conditions which affect humans and a wide variety of animals. Prion diseases are also called transmissible spongiform encephalopathies (TSEs), a term that underlines their infectious character. The most widely accepted hypothesis on the nature of the infectious agent causing TSEs (the prion) predicates that it consists essentially of PrP^{Sc}, an abnormally folded, protease-resistant, β-sheet rich isoform of a normal cellular protein termed PrP^{C}. Its gene, *Prnp*, was identified more than a decade ago by Charles Weissmann, and shown to encode the host protein PrP^{C}. Since the latter discovery, transgenic mice have contributed many important insights into the field of prion biology. By disrupting the *Prnp* gene, it was shown that an organism that lacks PrP^{C} is resistant to infection by prions. Introduction of mutant PrP genes into PrP-deficient mice was used to define the structure-activity relationship of the PrP gene with regard to scrapie susceptibility. Ectopic expression of PrP in PrP knockout mice proved a useful tool for the identification of host cells competent for prion replication. On the other hand, utilization of transgenic mice with distinct deficiencies in the immune system, such as a lack of B cells, facilitated the discovery of the involvement of the immune system, i.e. the lymphoreticular organs, in prion pathogenesis. Likewise, transgenic mouse models significantly helped to identify a second phase of prion neuroinvasion following colonization of lymphoreticular organs that involves peripheral nerves and the autonomic nervous system. Finally, it again was a transgenic mouse expressing anti-prion antibodies to deliver a proof-of-principle that a humoral immune response against the prion protein can antagonize prion infection. Thus, genetically modified mice are to date a powerful and indispensable tool (i) to delineate mechanisms by which peripherally administered prions invade the brain and ultimately provoke damage as well as (ii) to identify possible preventive and/or therapeutic approaches to prion diseases.

31 Parkinson's and Alzheimer's disease in *C. elegans*

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We use *C. elegans* to study the genetics and function of genes associated with hereditary forms of neurodegenerative diseases. (A) Alzheimer' Disease (AD): The *C. elegans* genome contains two presenilins, hop-1 and sel-12 that are required throughout the life of the animals. We have shown that FAD mutations in sel-12 result in a loss of presenilin function, which can be rescued by expression of human PS1 or PS2 in *C. elegans*. We identified mutations in five gene loci (spr modifiergenes: suppressors of presenilin defects) that suppress the *C. elegans* FAD defects. All spr mutants have a similar phenotype: they de-repress the transcription of hop-1, and HOP-1 protein is able to substitute for the defective SEL-12 in the γ-secretase complex. Our dat suggest that familial

forms of AD should be treated by upregulating, rather than inhibiting, presenilin activity. The spr modifier genes may, thus, serve as new target for AD treatment.

(B) Parkinson`s Disease (PD). Mutations in the parkin gene cause autosomal recessive juvenile Parkinsonism (AR-JP), an inherited form of PD with very early onset. Several labs have constructed parkin knock-out strains in mouse, yet none of these models revealed any phenotype. We identified and characterized the *C. elegans* orthologue of human parkin, which we named pdr-1 (Parkinson`s Disease related). PDR-1 shares with parkin the same architectural structure, with 28 % identity and 41 % similarity on protein level. Parkin/PDR-1 is a RING-containing E3 ubiquitin-protein ligase required for ubiquitin-dependent targeting and proteasomal degradation of specific protein substrates. We isolated three deletion mutants of pdr-1. Using a combination of genetic and biochemical methods, we identified a crucial role of pdr-1 in a pathway conserved from nematodes to humans. Loss of pdr-1 can be compensated by transgenic rescue. Using yeast interaction studies and GST-pulldown experiments, we characterized protein interactions and enzymatic activity of the PDR-1 protein. We will present data showing that it interacts with ubiquitin-conjugating enzymes (E2) and with the *C. elegans* orthologue of CHIP. This is to our knowledge the first animal model of parkin function.

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Inducible neuronal expression of TGF-β1 in transgenic mice 32

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Background: Transforming Growth Factor-β (TGF-β1) is involved in the pathogenesis of Alzheimers Disease (AD). The expression of TGF-β1 is increased in brain tissue of AD patients and elevated levels have been reported for the cerebrospinal fluid. TGF-β1 is associated with accelerated deposition of β amyloid in amyloid precursor protein transgenic mice and thus may promote or initiate β amyloid accumulation in senile plaques of AD patients. Additionally, TGF-β1 seems to influence the clearing of β amyloid. TGF-β1 can be synthesised and released by astrocytes or microglial cells as well as neurons. Different transgenic mice models have been generated by others expressing TGF-β1 by astrocytes in a constitutive manner. The disadvantage of these models is the uncontrolled local and temporal TGF-β1 overexpression. Furthermore, the neuronal TGF-β1 synthesis observed after stress or in AD is not modulated by transgene expression in astrocytes. Objective and Methods: We established an inducible neuron-specific transgenic mouse model based on the tetracycline system where the expression of TGF-β1 can be regulated by administration of doxycycline. Results and Conclusions: TGF-β1 is expressed in hippocampal and cortical neurons, affecting both neuronal and astrocytic homeostasis and extracellular matrix proteins.

33 Altered expression of plasticity-related genes in syn-ras transgenic mice

Ellen Ramminger [1], Uwe Ueberham [1], Annette G. Beck-Sickinger [2], Rolf Heumann [3] and Thomas Arendt [1]

[1] Neuroanatomie, Paul-Flechsig-Institut für Hirnforschung, Jahnallee 59, 04109 Leipzig, Germany; [2] Biochemie und Bioorganische Chemie, Institut für Biochemie, Talstrasse 33, 04103 Leipzig, Germany; [3] Molekulare Neurobiochemie, Fakultät für Chemie, NC 7/174, 44780 Bochum, Germany

Neurodegeneration in Alzheimer's disease is associated with activation of mitogenic signaling mediated through the small G-protein p21ras that drives activation of the Π3 pathway and the MAPkinase cascade. A transgenic mouse model that expresses permanently activated human ras ($p21ras^{Val12}$) under control of the synapsin-promotor specifically in neurons was used to characterize the pathogenic chain and identify potential therapeutic downstream targets. Differences in the transcriptom compared to wildtype were analyzed by DNA-microarray (Affymetrix®). Expression of 10 genes of interest was specified further by quantitative one-step RT-PCR (rotor gene®). Major changes were identified in the expression of genes coding for proteins known to be involved in synaptic plasticity such as synaptogyrin 1b, calcium calmodulin dependent proteinkinase II α (CAMKII α) and MAP2. The present results demonstrate alterations in the expression of plasticity-related genes in a mouse model of Alzheimer's disease and support a critical link between neuroplasticity and neurodegeneration.

34 A caged doxycycline analog for photoactivated gene expression with high spatiotemporal resolution

Sidney Cambridge, Beate Cürten and Tobias Bonhoeffer

Max-Planck-Institute of Neurobiology, Germany

We have established a system that allows induction of transgene expression in a defined set of cells by irradiation with UV light. To this end, we developed a photoactivatable version of the inducible tetracycline (tet) system (Tet-on) pioneered by Bujard and coworkers. A more potent analog of tetracycline, doxycycline was reversibly inactivated or "caged" to be used for photoactivated gene expression. Upon irradiation with UV light, doxycycline is released in its unmodified and biologically active form. By confining irradiation to a small area, gene expression can be induced with unprecedented spatial resolution, possibly in single cells.

Caged doxycycline was synthesized and purified to homogeneity as determined by HPLC analysis. To assess the transcriptional activity of caged versus photoactivated doxycycline, CHO cells expressing a tet-dependent EGFP construct were used as a simple cell culture assay. Irradiation of cells that were incubated with caged doxycycline displayed widespread EGFP fluorescence while cells in the same dish that were not irradiated did not fluoresce. Doses of UV light necessary for uncaging appeared to be non-toxic as cells in irradiated areas did not show signs of necrosis.

A second assay to test photoactivation in organotypic hippocampal Müller cultures was also employed. Two adenoviruses, one constitutively expressing the transactivator and one containing a tet-dependent chloramphenicol transferase (CAT) construct, were used to infect wild-type mouse brain slices. After administration of caged doxycycline and subsequent photoactivation, CAT expression was detected in irradiated slices but not in the unirradiated control. Furthermore, local irradiation of slices produced only local expression of CAT.

Our results clearly demonstrate, that caged doxycycline can be used to induce transgene expression in defined cells making it a powerful tool for biomedical research.

Introductory Remarks to Symposium 5

Signal integration in dendrites

Thomas Berger and Matthew Larkum

The last decade has seen a resurgence of interest in the properties of dendrites, spurred on by advances in techniques that have allowed researchers to probe their active nature. Up to the beginning of the 1990's it was fashionable to treat dendrites as passives structures in order to reduce their complexity and allow predictions of what computational advantage dendrites might provide. More and more since this time, researchers and theoreticians have had to face up to the additional complexity represented by dendrites with active conductances. Within this new framework there have been two paradigms: the one, treating dendritic conductances as mechanisms to compensate for the passive effects of dendrites and thereby normalize the efficacy of synaptic contacts over the whole tree, and the other, treating dendritic conductances as crucial for additional computational capabilities only possible with interactions in the dendritic tree.

Signal integration in active dendrites is enriched by two features that have been found in most neuronal cell types studied so far: a) action potentials can propagate actively along dendrites (the notable exception being Purkinje cell dendrites) and b) dendrites have regenerative regions which can produce local and/or forward propagating action potentials. Thus, signal integration from the modern perspective must embrace the concept that cells can send information about activity from one subcellular region to another. Furthermore, additional mechanisms can come into play for modulating this form of intracellular communication. This leads to a much more complicated view with signal integration being distributed in place and time throughout the whole cell.

The talks in this symposium will cover the recent research into these topics. Alex Reyes will demonstrate some of the properties of synaptic integration above and below threshold for action potentials. Then Jeff Magee and Matthew Larkum will introduce the topic of dendritic action potentials and active propagation in hippocampal and neocortical pyramidal cells. The second session will concentrate on the modulation of intracellular communication and the consequences for signal integration by inhibitory synaptic inputs (Michael Häusser) and leak conductances (Thomas Berger). Lastly, Greg Stuart will show how dendritic interactions can modulate calcium influx through NMDA channels.

35 Integration of Synaptic inputs: Summation in the subthreshold and suprathreshold ranges

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To generate firing, neurons must integrate the many synaptic inputs that originate in their dendrites. This process is complicated by the fact that neurons have numerous voltage- and time-dependent conductances, some of which are distributed non-uniformly throughout the dendritic tree. Thus, whether summation is linear or non-linear will depend in part on the number, timing, and location of the synaptic inputs. Here, we systematically examine how these variables affect the summation of postsynaptic potentials (PSPs) and their associated changes in firing rate. First, to examine how unitary PSPs sum, we perform simultaneous whole-cell recordings from a layer 5 pyramidal neuron and two presynaptic layer 3 pyramidal cells. Then, to examine summation of multiple inputs, we record simultaneously from two dendritic sites and, with the aid of a computer, inject current that would be generated by a population of presynaptic neurons. Finally, to examine summation in the suprathreshold range, we increase the number of injected PSPs until the neuron fires repetitively. Preliminary results indicate that unitary PSPs, as well as individually injected PSPs, sum linearly. However, as the number of injected PSPs is increased, summation becomes supralinear. In the suprathreshold range, summation is initially supralinear -the firing rates caused by two dendritic inputs are greater than the sum of their individual effects- but becomes sublinear as the number of injected PSPs increases. We are currently examining the possible mechanisms that underlie these observations.

36 Regulation of local dendritic spike initiation and propagation in CA1 pyramidal neurons.

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The presence of voltage-gated ion channels in the apical dendrites of CA1 pyramidal neurons allow axonally-initiated action potentials to propagate back into the dendrites. These same channels can, under appropriate conditions, also allow spikes to be initiated locally within the arborization itself. These local dendritic spikes usually decrement very rapidly to arrive at the soma as small spikelets or simply act to boost the underlying EPSPs that evoked the spike. We have used dual whole-cell recordings and Fura-2 fluoresence imaging to investigate the regulation of local spike threshold, peak spike amplitude and propagation distance. Preliminary data suggests that under control conditions a short train of large amplitude EPSC-shaped current injections can evoke dendritic spikes of variable amplitude that actively propagate for approximately 100 um before becoming too small to activate dendritic Ca^{2+} influx. The threshold (current and voltage), amplitude and propagation distance of these local spikes were modified by physiologically-relevant changes in extracellular $[K^+]/[Ca^{2+}]$ and by the application of various neuromodulators (5-HT and NE). These results have significant implications for the

regulation of synaptic integration within the arbors of CA1 pyramidal neurons (supported by NIH and NSF).

Dendritic interactions in layer 2/3 neocortical pyramidal neurons

37

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Pyramidal neurons are the most abundant cell type in the neocortex. Their stereotypic morphology with dendritic aborizations proliferating in specific layers suggests that they receive functionally different inputs to specific regions of the cell. Within the pyramidal neuron class, layer 2/3 pyramidal neurons are by far the most numerous and it has been hypothesized that they bear the major responsibility for the local computation performed within a cortical column. They receive ascending sensory input from layer 4 which arrives mostly on the basal dendrites. In addition, they receive descending feedback input from layer 1 fibers which terminate mostly in the distal tuft region. Much less is known about the active properties of the dendrites of L2/3 pyramidals than L5 pyramidals. Specifically it is not known whether they can perform the same kinds of associational computations using backpropagation activated calcium spike firing (BAC firing).

Here we show that backpropagating APs and dendritic depolarization also interact in an associative manner in L2/3 neurons. Neither a single backprogating AP alone nor a short subthreshold injection of current into the distal dendrite causes influx of calcium into the tuft region. In combination, however, threshold is reached for a suprathreshold distal potential and a substantial influx of calcium occurs. We showed that this is also the case when the dendritic current injection is replaced with a compound distal EPSP evoked by extracellular stimulus of the layer 1 fiber tract in the presence of inhibitory antagonists. It could also be evoked using coincident extracellular stimulation of layer 1 and layer 4 under the same conditions. The interaction of the inputs is highly time-dependent with a similar time course to L5 pyramidal neurons. The suprathreshold dendritic event in L2/3 pyramidal neurons was much shorter than the equivalent potential in L5 pyramidal neurons which may reflect a lower density of calcium channels in the tufts of L2/3 neurons. We could also induce a large dendritic depolarization and calcium influx with bursts of 4 somatically evoked APs at high frequencies (>~120 Hz). The transition from low to high calcium influx occurred over a wider range of frequencies in L2/3 pyramidal neurons than in L5 pyramidals and the threshold was slightly higher. Distal calcium influx was also observed *in vivo* (using 2-photon microscopy) showing that AP activity can lead to regenerative events in the distal dendrites in the intact brain. We conclude that L2/3 neurons can use active dendritic properties to associate basal (ascending sensory) and distal (feedback) synaptic inputs.

38 Interactions of Action Potentials with Somatic and Dendritic IPSPs

Michael Häusser

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Action potentials (APs) and inhibitory postsynaptic potentials (IPSPs) are interacting continuously in the intact brain. The outcome of this interaction determines the input-output relationship of the neuron. To investigate the nature of this interaction, we have paired antidromic APs at various times relative to somatic or dendritic IPSPs generated using the dynamic clamp technique. Subtracting the antidromic AP obtained in isolation from the combined IPSP/AP waveform revealed that the IPSP was substantially shunted by the AP. Shunting was dependent on the relative timing of the IPSP and AP, being greater when the AP occurred during the IPSP than when the AP preceded it. Comparison of dynamic clamp and current synapses shows that shunting is mitigated by a transient increase in IPSP driving force during an AP. Somatic IPSPs are shunted more strongly than distal IPSPs at all time points, for both dynamic clamp and current synapses. Simulations in a detailed compartmental model indicate that this is because dendritic inhibitory synapses are 'protected' from the large AP conductances concentrated in the axon. Finally, shunting is less pronounced than for EPSPs, suggesting that the balance between excitation and inhibition may be influenced by the firing rate.

39 Electrotonic Separation of Two Spike Initiation Zones in Layer 5 Pyramidal Cells of the Somatosensory Cortex: Role of the Hyperpolarization - Activated Current I_h

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The layer 5 pyramidal cell of the somatosensory cortex operates with two spike initiation zones. A low-threshold sodium spike is initiated in the initial segment of the axon, whereas a high-threshold calcium spike may be generated in the distal apical dendrite. The influence of dendritic synaptic potentials on somatic spike generation as well as the importance of somatic spikes for dendritic integration depends critically on the electrotonic distance between both zones. In recent work we could show that the deactivation of the hyperpolarization-activated current (I_h) leads to the attenuation of sub-threshold synaptic potentials. Thereby it reduces the importance of distal synaptic input for action potential generation. In the present work, we were interested if I_h could in addition influence the generation of supra-threshold calcium events in the distal dendrite due to somatic spikes. In acute slices of the rat somatosensory cortex, layer 5 pyramidal cells were studied under current-clamp. Whole-cell recordings were obtained from the soma or from the soma and the dendrite. Four consecutive action potentials were induced with different inter-spike intervals by short current injections into the soma. When these intervals reach a critical frequency (CF), calcium current - mediated events were seen in the dendrite (CF = 105.7 Hz; n = 52). These calcium potentials were blocked by combined application of 100 µm $NiCl_2$ and 100 µm $CdCl_2$ (n = 3). The CF depends on the presence of Ih: Blockade with 20 µm ZD7288 reduces the CF to 68.2 % of control

(n = 11). Serotonin, isoproterenol, or dopamine did not influence the CF, presumably due to the presence of cAMP-insensitive Ih channels in the cell type under study (n = 12 in total). However, depolarization in the dendrite reduced the CF, while hyperpolarization increased it. These data suggest, that the hyperpolarization - activated current influences not only the dendro-somatic but also the somato-dendritic interaction and separates electrotonically the two spike initiation zones in layer 5 pyramidal cells.

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Dendritic mechanisms involved in spike-timing dependent plasticity 40

Greg Stuart and Bjoern M. Kampa

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Coincidence of action potentials (APs) and EPSPs is known to be important for the induction of many forms of synaptic plasticity. The mechanism responsible for this is thought to involve NMDA receptor activation following relief of Mg^{2+} by backpropagating APs. Here we directly investigate this using the activity-dependent NMDA channel blocker MK801. Pairing of single APs with NMDA EPSPs did not increase MK801 block, whereas a burst of 3 APs evoked just after EPSP onset (+10 ms) increased MK801 block two-fold. Surprisingly, AP bursts evoked prior to EPSP onset (-15 ms) lead to a similar degree of MK-801 block, whereas bursts evoked 35 ms before EPSP onset slightly reduced MK801 block. Consistent with the role of NMDA activation in synaptic plasticity, EPSP-AP pairing at both +10 and -15 ms induced LTP whereas pairing at –35 ms induced LTD. Membrane patches were used to directly monitor NMDA channel activation during APs. These experiments indicated that relief of Mg^{2+} block by somatic AP bursts evoked only small NMDA currents, whereas the dendritic response to AP bursts caused substantial NMDA activation. A kinetic model of NMDA channels with realistic rate constants for Mg^{2+} block and unblock indicated that bursts of dendritic APs trigger large NMDA-mediated Ca^{2+} currents. These results demonstrate that dendritic electrogenesis associated with AP bursts can, if timed appropriately, significantly increase NMDA receptor channel activation and so dendritic calcium influx. This finding presumably underlies the importance of burst firing for induction of synaptic plasticity in the cortex.

Biophysical Properties and Distribution of Large-conductance Calcium-dependent Potassium Channels in Neocortical Layer 5 Pyramidal Neurons. 41

Narimane Benhassine and Thomas Berger

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Layer 5 pyramidal cells in the somatosensory cortex are characterized by two action potential initiation zones, one in the soma and one in the apical dendrite. Because calci-

um-activated high-conductance potassium channels (BK channels) on the dendrite could critically modulate the interaction between both zones, properties and distribution of the BK channels were studied in an acute slice preparation of rat somatosensory cortex. Recordings were obtained from layer 5 pyramidal neurons using the patch-clamp technique in the inside-out and outside-out configuration. Due to their large conductance, BK channels could be easily distinguished from other potassium channels. BK channels were characterized by a conductance of 138.5 ± 11.8 pS (mean ± S.D.) at room temperature and by their calcium dependence. In addition, BK channels were blocked by 1 mM TEA and 3 μm of the specific blocker paxilline. Their opening probability increased due to depolarization and increasing calcium concentration on the inner side of the membrane. The opening probabilities were 28, 27, 87, and 78 % at -100, -50, +50, and +100 mV respectively. In addition, the mean channel open and closed time constants were voltage-dependent. Two exponential functions were required to describe the distribution of channel open times at depolarized potentials, suggesting two different open channel states. At hyperpolarized potentials, in contrast, one exponential function was sufficient. The time constants were as follows: τ_{open1}, 0.3, 0.6, 1.3, and 2.8 ms at -100, -50, +50, and +100 mV, respectively; τ_{open2}, 16.0 and 28.8 ms at +50 and +100 mV, respectively. The closed state time constant (τ_{closed}) was 4.6, 4.6, 0.8, and 1.6 ms at -100, -50, +50, and +100 mV, respectively. Finally, the distribution of these channels along the somato-dendritic axis of the layer 5 pyramidal cell was of special interest. BK channels were found either isolated or in clusters containing up to four channels (mean: 1.8 channels per patch; n = 81). Recordings were performed at the soma and along the dendrite up to 850 μm from the soma. Surprisingly, the BK channel density distribution was found to be homogeneous along the dendrite (Kendall´s statistical test, $\tau = 0.08$). This study presents the first evidence for the presence of BK channels on dendrites. BK channels might be critical for the following modulatory processes: i. shortening the action potential, ii. limitation of its backpropagation into the dendrite, and iii. increase in the electrotonic distance between the two action potential initiation zones.

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42 The gain of L5 pyramidal neurons is larger for distal than for somatic input

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Inputs arriving at distal locations on the dendrites of L5 pyramidal neurons are severely attenuated and are therefore expected to have much less impact on firing than proximal inputs. Our experiments show that the reverse is true. We analyzed the frequency-current (f/I) relationships to simultaneous in-vivo-like current inputs at different somato-dendritic compartments. We found that distal current input have up to 4 times higher gain. The effect is due to calcium conductances activated by conjunct dendritic depolarization and back-propagating action potentials leading to burst firing. The timing of these bursts codes the presence of coincident somatic and dendritic inputs within a

narrow time window. A simple 2-compartment intgrate-and-fire model is necessary and sufficient to account for these effects. We suggest to look at L5 pyramidal cells as 2-compartment units which can operate in a sub- and supra-(calcium-)threshold mode. In the sub-threshold mode the influence of the dendritic compartment is actively suppressed. In the supra-threshold mode, however, the dendritic compartment dominates the neuronal firing behavior. In this mode, dendritic gain modulation and timing of bursts allows the neurons to associate top-down and bottom-up input.

Dendritic mechanisms involved in spike-timing dependent plasticity

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Coincidence of action potentials (APs) and EPSPs is known to be important for the induction of many forms of synaptic plasticity. The mechanism responsible for this is thought to involve relief of Mg^{2+} block of synaptic NMDA receptors by backpropagating APs. Here we directly investigate this using the activity-dependent NMDA channel blocker MK801. Pairing of single APs with NMDA EPSPs did not increase MK801 block, whereas a burst of 3 APs evoked just after EPSP onset (+10 ms) increased MK801 block two-fold. Surprisingly, AP bursts evoked prior to EPSP onset (-15 ms) lead to a similar degree of MK-801 block. Consistent with the role NMDA activation by backpropagating APs in synaptic plasticity, EPSP-AP pairing at both –15 and +10 ms induced LTP. Membrane patches were used to directly monitor NMDA channel activation during APs. These experiments indicated that relief of Mg^{2+} block by somatic AP bursts evoked only small NMDA currents, whereas the dendritic response to AP bursts caused substantial NMDA activation. A kinetic model of NMDA channels with realistic rate constants for Mg^{2+} block and unblock indicated that bursts of dendritic APs trigger large NMDA-mediated Ca^{2+} currents. These results demonstrate that dendritic electrogenesis associated with AP bursts can, if timed appropriately, significantly increase NMDA receptor channel activation and so dendritic calcium influx. This finding presumably underlies the importance of burst firing for induction of synaptic plasticity in the cortex.

Inhibition of back-propagating spikes in a cerebellum-like sensory structure in the weakly electric fish *Gnathonemus petersii*, by a general anaesthetic

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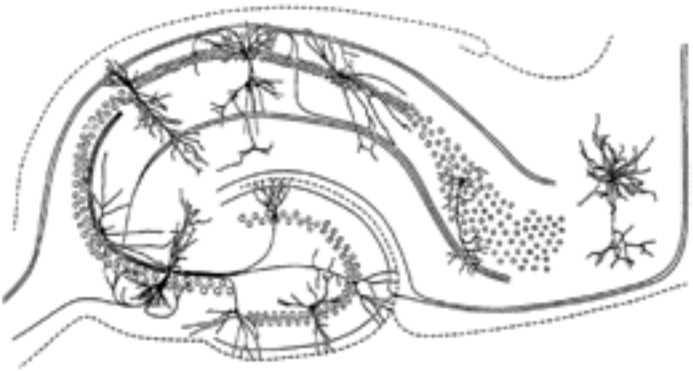
Many *in-vivo* studies of neural activity make use of anaesthetised animals. Anaesthetics by themselves, however, may induce specific neural changes that alter the balance of

activity within neuronal networks, thus biasing the outcome of physiological experiments. The general anaesthetic etomidate, a potentiator of GABAergic activity at $GABA_A$-receptors, has recently been introduced in in-vivo studies of the electrosensory lateral line lobe (ELL) of the mormyrid *Gnathonemus petersii*. Unlike other anaesthetics, etomidate preserves the electric organ discharge, so that electrosensory processing in the ELL can be studied using a natural stimulus in this active sensorimotor system.

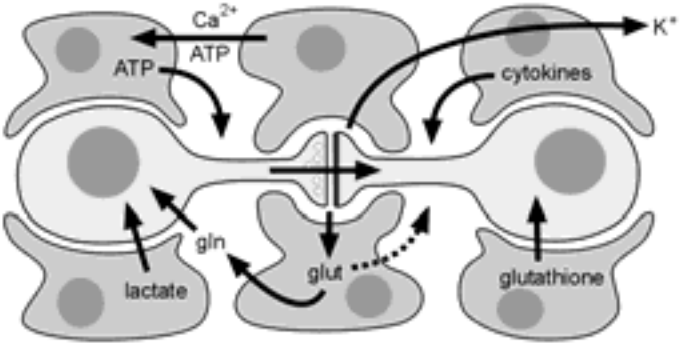
Here we describe the effects of etomidate on the neural network of the ELL as determined by both extra- and intracellular recordings, *in vivo* as well as *in vitro*. Field potentials and current source density analyses *in vitro* indicate that etomidate reduces the probability of back-propagating spikes through the ELL molecular layer, composed in majority by the apical dendrites of Medium Ganglionic (MG) GABAergic interneurons. In the presence of the $GABA_A$-receptor blocker bicuculline, etomidate was without effect, and the interneurons seemed more active.

Consistent with the field potential changes observed *in vitro*, preliminary results from *in-vivo* experiments on curarised fish (using an artificial electrosensory stimulus) show that etomidate induces dose-dependent, reversible field potential changes in the ganglionic layer of the ELL, further indicating that the response of the interneurons to an electrosensory stimulus is modulated by the anaesthetic. The effects of the related molecule metomidate appear to be similar.

We speculate that etomidate modulates the balance of activity in intrinsic GABAergic microcircuits in ELL and may potentiate lateral inhibitory mechanisms. Currently intracellular experiments are in progress, *in vivo*, to examine the effect of etomidate on the modulation of inhibitory receptive fields and the role of modulation of inhibitory circuits on network plasticity which previous studies have linked to the presence of back-propagating action potentials.



Reactive changes of glial functions -



Beneficial or detrimental?

Introductory Remarks to Symposium 6

Neuronal death and neuroprotection: The role of glial cells

Andreas Reichenbach and Christian Steinhäuser

It is well known that glial cells in the brain undergo distinct and characteristic morphological alterations under pathological conditions. Evidence is now accumulating that the structural changes are accompanied by variations in glial functioning. These cells express a set of ion channels and receptors similar to their neuronal counterparts, and alterations of gating properties or expression levels of these channels and receptors might be involved in pathological processes of the CNS.

The Symposium aims at demonstrating that alterations in functional and molecular properties of microglia, oligodendrocytes and astrocytes can be causative of various CNS diseases, or, by contrast, exert protective effects. Six glia experts from four different countries will summarize latest knowledge indicating a critical role of glial cells in demyelinating disorders, epilepsy, and in the diseased retina.

Two lectures address the impact of glial cells in epilepsy. C. Steinhäuser describes functional and molecular changes of astroglial ionotropic glutamate receptors (AMPA subtype) and inwardly rectifying K^+ channels in the hippocampus of patients suffering from temporal lobe epilepsy. This subject is picked up by J. Gorter who provides a complementary report on altered expression of Na^+ channels and metabotropic glutamate receptors in microglia and astrocytes in a rat model of epilepsy. Both authors suggest a role for glial cells in seizure generation and/or seizure spread in the epileptogenic hippocampus. P. Kofuji shows that glial K^+ channels are crucial for oligodendrocyte development and myelination, supposing that glial cells are critically involved in the pathogenesis of demyelinating diseases. A. Buisson has identified glial factors secreted in response to TGF-beta stimulation which mitigate excitotoxic neuronal death. In his talk he will explain how these glial factors reduce the activation of glutamate receptors and why they might represent interesting candidates for alternative approaches to neuroprotection. A. Reichenbach summarizes recent findings on retinal Müller cells, delineating metabolic pathways enabling the cells to exert protective effects against glutamate-mediated neurotoxicity and free radical-induced injury. Finally, T. Reh presents evidence for the possibility of glia-derived neuronal repair. His data indicate that gliotic Müller cells constitute a source of neuronal transdifferentiation in the postnatal retina.

The speakers of this Symposium will provide compelling evidence that astrocytes, oligodendrocytes and microglia are highly plastic cell types that undergo various, parallel functional changes in the course of a disease. Promising approaches in analysing the role of glial cells in pathogenesis have to consider these multiple mechanisms, as well as sub-regional peculiarities defined by the cell's specific microenvironment.

45 Functional and molecular changes in astrocytes of human epileptic hippocampus: Relevance to seizure generation

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Functional and molecular properties of astrocytes were investigated in human hippocampi resected from patients with intractable temporal lobe epilepsy (TLE). Histopathological analysis distinguished two forms of epilepsies, Ammon's horn sclerosis (AHS) and lesion-associated TLE, with the latter resembling normal control tissue. Patch-clamp recordings were obtained from astrocytes in the CA1 stratum radiatum. All cells responded with receptor currents upon fast application of glutamate or kainate. Cells from AHS cases possessed a slower desensitization kinetics of glutamate responses and a stronger cyclothiazide-mediated potentiation of current amplitudes, suggesting an up-regulation of AMPA-R flip splice variants in astrocytes of the sclerotic tissue. Multiplex and semiquantitative single cell real-time RT-PCR subsequent to electrophysiological recordings identified enhanced expression of GluR1 flip in AHS. Activation of AMPA receptors caused a dose-dependent block of inwardly rectifying K^+ (Kir) channels. Hyperpolarisation of human astrocytes elicited Kir currents, but inward rectification was reduced in astrocytes from patients with AHS. Real-time RT-PCR demonstrated a downregulation of Kir4.1 underlying the reduction in current amplitudes. We conclude that in AHS, glutamate will lead to prolonged depolarization of astrocytes which probably contributes to generation and spread of seizure activity.

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46 Molecular and immunocytochemical changes in macro- and microglia in a rat model of mesial temporal lobe epilepsy

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Mesial temporal lobe epilepsy (MTLE) is characterized by neuronal cell death, gliosis and synaptic reorganization in specific limbic areas. Astrocytes and microglial cells are known to undergo morphological changes and to become reactive following a severe insult (status epilepticus). This leads to upregulation of various genes and proteins, some of which are involved in neuroprotection. Here we focus on glial changes at the molecular level in a rat model for MTLE. Remarkably, these changes can have opposite effects on the process of epileptogenesis. 1) Using serial analysis of gene expression on hippocampal tissue of rats 8 days after status epilepticus (latent period) we found that proteases (cathepsins) and an inhibitor of proteases (Cystatin-C) were strongly upregulated. Immunocytochemical studies confirmed increased and persistent expression in limbic regions not only in astrocytes and microglial cells but also in neurons. Upregula-

tion of Cystatin-C, which peaks at 1 week after SE, could be a mechanism by which the progression of damage is limited. 2) Using immunocytochemical methods, we found a strong upregulation of two metabotropic glutamate receptors subtypes (mGluR3 and mGluR5) in reactive astrocytes in the (para)hippocampal region. Increased expression of these glial mGluRs persisted in areas where cell loss was extensive (e.g. entorhinal cortex layer III, hilar region). Upregulation of glial mGluR3 could have neuroprotective effects, presumably mediated by release of TGF-β and other growth factors that are also upregulated in reactive glial cells (1 day - several weeks after SE). In the opposite direction, upregulation of glial mGluR5 subtype could contribute to seizure spread via Ca^{2+} waves, astroglial swelling and glial glutamate release.

Dystrophins, syntrophins and glial cell function in retina 47

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Absence of dystrophins in the retina is believed to cause alterations in electroretinograms such as decrease of b-wave amplitude. This alteration in the b-wave is seen both in human patients as well as in animal models (mdx 3Cv mouse) and has been atributed to the loss of the shorter isoform DP71 in glial cells. The reduced b-wave is indicative of an impairment of synaptic transmission between photoreceptors and bipolar cells. Recently we found that the dystrophin-associated protein complex (DAPC) is critical for localization of the inwardly rectifying potassium channel, Kir4.1 in Müller glial cells of retina. While in the wild type mouse the Kir4.1 is highly concentrated in the Müller cells endfeet and perivascular processes, in the mdx 3Cv mouse these channels are uniformly localized in the glial plasma membrane. A possible link between the DAPC and Kir4.1 is the PDZ-domain containing protein, α-syntrophin. We found that Kir4.1 is able to interact with the α-syntrophin from mouse brain lysates and cultured astrocytes. Furthermore Kir4.1 and α-syntrophin coimmunoprecipitate from transfected COS cells. This interaction is ablated when the PDZ binding domain on Kir4.1 is absent. These results suggest that Kir4.1 may be localized to particular subcellular domains of glial cells by the DAPC through a PDZ-mediated interaction with α-syntrophin. We suggest that the improper expression of Kir4.1 channels in Müller cells of the mdx 3Cv mouse leads to an impairment of photoreceptor-bipolar cell neurotransmission. These studies suggest that specific localization and clustering of transporters and ion channels in subdomains of glial cells is critical for their role in sustaining neuronal activity.

Can Glial cells modulate excitotoxic neuronal injury? 48

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Along with the fact that it is the predominant excitatory neurotransmitter in the mammalian brain, studies have shown that glutamate could play a pivotal role during hypoxic-ischemic insult. Ischemia-induced neuronal injury is thought to result from an

excessive release of excitatory amino acids and the subsequent activation of their post-synaptic receptors. This excitotoxic pathway may also contribute to brain and spinal cord cell loss after various acute insults. Accordingly, cell culture models of glutamate toxicity have been extensively used to investigate the mechanisms of neuronal injury associated with these pathologies. The first step in the excitotoxic cascade is mediated by the activation of ion channel-linked glutamate receptors, especially N-methyl-D-aspartate (NMDA) receptors, probably because of their high Ca^{2+} permeability. The subsequent activation of cytoplasmic calcium-dependent enzymes has been proposed to mediate the final excitotoxicity.

More recently, various clues have emerged to suggest that both global and focal ischemia may have an apoptotic component. This type of cell death is a process morphologically distinguishable from excitotoxicity and characterized by cell volume loss, membrane blebbing, chromatin condensation and DNA fragmentation. Apoptosis can be inhibited by inhibitors of either transcription or translation, results that suggest the expression of active “death proteins”.

A considerable ongoing effort is devoted to develop practical methods to attenuate both types of neuronal death during ischemia. The most direct approach to block excitotoxic brain injury during cerebral ischemia is to use glutamate receptor antagonists. However, because of the potential safety concerns of these compounds, including psychotomimetic effects and disruption of synaptic plasticity, there is an increasing interest for alternative therapeutic strategies. Because of their capacity to promote neuronal survival during embryogenesis, the use of growth factors has been proposed to limit the consequences of cerebral ischemia. Several studies have identified a number of cytokines produced by neurons and/or glia in response to insults to the neuropil. Among these cytokines, a structurally related family has gained prominence because of its high level of expression in biopsies of patients with acute brain injury or neurodegenerative diseases: the Transforming Growth Factors β. Even if the survival-promoting properties of growth factors against apoptosis have been extensively described, Transforming growth factor-β fails to affect apoptotic cell death in cortical neurones and can be characterized as a non-conventional growth factor. Here we describe the fact that the neuroprotective capacity of Transforming growth factor-β is restricted to the necrosis induced by N-methyl-D-aspartate and occurs only in the obligatory presence of Transforming growth factor-β responsive astrocytes. We demonstrate that this neuroprotective effect is mediated through a Transforming growth factor-β-induced up-regulation of a serine protease inhibitor in astrocytes, results which would indicate that the metabolism of the extracellular matrix may play a pivotal role in neuronal viability.

49 Muller cells protect neurons by transfer of glutathione, and by control of extracellular glutamate

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The retina, as a highly specialized part of the CNS, is characterized by several peculiarities which are potentially dangerous for the survival of neurons. Among those are (i) an

extremely active oxidative metobolism, with the accompanying unavoidable generation of free radicals and reactive oxygen species, (ii) a very high turnover rate of membrane lipids (which are very sensitive against free radicals), and (iii) the virtually continuous release of (potentially excitotoxic) glutamate by the majority of retinal neurons. This situation is even aggravated by the facts that most retinal neurons fail to contain significant amounts of endogenous free radical scavengers such as glutathione, and that neuronal glutamate uptake is rather slow. Thus, not only in cases of ischemia-reperfusion but even under physiological conditions, neuroprotective mechanisms may be challenged. Such neuroprotective mechanisms are provided by the dominant retinal macroglia, the Müller cells. These cells are known to be the main sites of retinal glutathione synthesis and storage, and to possess very efficiant glutamate uptake carriers.

In a first series of experiments, we examined the possibility that Müller cells provide a glutathione support of neurons in acutely isolated mammalian retinae. Pieces of acutely isolated retinae from rats and guinea pigs were incubated for various time periods with different concentrations of hydroperoxide or tert-butylhydroperoxide (t-BHP). The thiol-reactive vital dye, CellTracker Green CMFDA, was used to assess intracellular levels of reduced glutathione (GSH) by means of a confocal laser scanning microscope. In control retinae, the GSH-sensitive-dye was exclusively detected in Müller cells, whereas the ganglion cells were devoid of fluorescence. After application of hydroperoxide or t-BHP, the GSH levels in Müller cells decreased, and the ganglion cells became fluorescent. This shift of fluorescence from Müller cells to ganglion cells was gradual, and depended on the duration and intensity of the oxidative stress. It was not affected by an inhibition of the γ-glutamyltranspeptidase or by a block of the GSH synthesis with buthioninesulfoximine. Removal of the extracellular sodium ions during the peroxid application prevented the increase of intracellular GSH in ganglion cells, but not the decrease of intracellular GSH in Müller cells. We conclude that under oxidative stress, a transfer of intact glutathione (oxidized and/or reduced) molecules occurs from Müller cells to ganglion cells. Furthermore the data are compatible with the idea that sodium-dependent transporters are involved in the GSH uptake of ganglion cells but not in the GSH release from Müller cells.

Earlier we had shown that glutamate uptake is crucial precondition for GSH synthesis in Müller cells, suggesting that glial glutamate uptake plays a double role in retinal neuroprotection. In a second series of experiments, we thus examined the glutamate uptake in Müller cells. We found there seems to exist an alternative, Na^+-independent mode of glutamate uptake in Müller cells which may exert neuroprotective effects even if the cells are depolarized and their dominant Na^+-dependent uptake carriers are inhibited.

Microglia: Friend or Foe? 50

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The role of glial cells is to support, sustain and if necessary restore proper neuronal function. Microglial cells, the resident immune cells of the central nervous system (CNS) are no exception to this. They have been implicated in many acute and chronic neurological diseases, including trauma, stroke and multiple sclerosis. Upon CNS injury

microglial cells are rapidly activated and transform from resting into activated microglia. Through the release of bioactive substances such as nitric oxide, cytokines and chemokines activated microglial cells can exert powerful toxic as well as protective effects on neurons and surrounding macroglia. Until recently research has mainly focused on the cytotoxic activation of microglial cells. Nevertheless newly emerging and revived older concepts challenge this view. Indeed, microglia play a crucial role in neuroprotection and neuroregeneration in a variety of insults. On the other hand microglial dysfunction in an otherwise unperturbed CNS might lead to neurodegeneration. How does the brain balance this dichotomy? From where we stand now, it is clear that microglial cells have a very strong neurotoxic potential. Once better understood, the neuroprotective and neuroregenerative properties could be manipulated to implement new therapeutic strategies.

51 Complete lack of gap junctional coupling in a subpopulation of astrocytes, termed GluR cells, in the hippocampus.

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We have identified two subpopulations of astrocytes coexisting in the hippocampus with distinct morphological and functional properties, specifically a non-overlapping expression of either AMPA-type glutamate receptors (GluR cells) or glutamate transporters (GluT cells) (Matthias et al., J Neurosci, 2003, in press). Gap junctional communication between astrocytes is thought to play a role in buffering of ions and metabolites and in astrocytic intercellular communication. Extending functional characterization of the two cell types, we tested whether they display the same amount of gap junctional coupling. GluR cells and GluT cells were identified in transgenic mice expressing EGFP under the control of the human GFAP promoter. They can be distinguished according to the pattern of EGFP-fluorescence. In the CA1 stratum radiatum of hippocampal slices, single fluorescent cells were filled with biocytin via the patch pipette during a 20 min period of whole-cell recording. After tissue-processing, the extent of tracer coupling was assayed quantitatively. Biocytin-filling of GluT cells led to an extensive spread of the tracer to neighboring cells. Usually, more than 100 labelled cells could be counted. In contrast, when a GluR cell was filled, the tracer was always confined solely to the recorded cell. No cell displayed an intermediate coupling pattern. To exclude the possibility of asymmetric coupling (allowing transfer in the GluT cell - GluR cell direction only) we further used a protocol for biocytin-visualization which preserved the internal EGFP-fluorescence. Identified GluR cells were never biocytin-positive after obtaining a cluster of tracer-coupled cells by GluT cell injection.

This study substantiates that the two subpopulations of EGFP-positive cells represent distinct cell types with contrasting physiological properties. As gap junctions are thought to play a role in inter-astrocytic Ca^{2+}-wave-propagation, and because GluR cells do not appear to be part of the astrocytic gap junctional syncytium, the communication among these cells might occur via different mechanisms, possibly the ATP/P2Y receptor pathway.

Functional NMDA receptors in post-ischemia astrocytes – a possible synaptic target? 52

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N-Methyl-D-Aspartate (NMDA)-type glutamate receptors play a critical role in neuronal communication, synaptogenesis, plasticity and excitotoxic death. Using immunohistochemistry, we detected NR2B subunit of the NMDA receptor in CA1 and subicular astrocytes of the hippocampus following ischemia-induced neuronal death in these regions. NR2B expression was first observed 3 days after ischemia and reached a peak at 28 days. At 56 days, only a few NR2B-expressing astrocytes were still present. *In vitro*, subjecting postnatal hippocampal cultures to 5 min of anoxia resulted in NR2B expression on astrocytes in the glial feed layer. Imaging of intracellular calcium using post-anoxia cultures and astrocytes acutely isolated from the ischemic hippocampus, revealed a rise in $[Ca^{2+}]i$ following stimulation with the specific agonist NMDA. The response could be reversibly blocked with the competitive antagonist 2-amino-5-phosphonovalerate and attenuated by the NR2B-selective antagonist ifenprodil.

We furthermore report first evidence for the possibility of synapse formation with reactive astrocytes: Confocal microscopy revealed an increase of presynaptically localized synaptophysin in the ischemic CA1 and the presence of synaptophysin puncta on the fine processes of astrocytes following ischemia. We detected neuroligin, a postsynaptic marker associated with excitatory synapses, in post-ischemia astrocytes, indicating that reactive astrocytes may receive direct synaptic input.

Kir channels in the hippocampus: Different expression in distinct types of astrocytes and alterations under pathophysiological conditions 53

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Astrocytes in the CNS possess inwardly rectifying K^+ (Kir) channels characterized by a high open probability at rest. These channels are presumably involved in extracellular K^+ buffering. Here, astroglial Kir currents were investigated with the patch clamp technique in the mouse hippocampus (CA1 stratum radiatum) *in situ*. To get information on putative Kir subunits expressed by these cells, immunocytochemical and single-cell transcript analysis (RT-PCR) was performed after electrophysiological recording. At

p12, Kir4.1 mRNA was expressed only by about 20 % of the cells tested, while subunits of the Kir2 family were found much more frequently. Recent work identified two functionally and morphologically different types of astrocytes, named GluR and GluT cells (Matthias et al., 2003, J Neurosci., in press). The above studies focused on astrocytes of the GluR-subtype, characterized by the expression of prominent voltage-dependent K^+ currents and ionotropic glutamate receptors (AMPA-subtype). We extended investigation to astrocytes of the GluT-type, possessing large time- and voltage-independent K^+ currents and functional glutamate transporters. In these cells, Kir4.1 was encountered more frequently as compared with GluR cells (50 vs. 20 %). To test for a contribution of different Kir subunits to astroglial Kir currents, we took advantage of transgenic mice with Kir4.1 (-/-) deletion. In astrocytes obtained from these mice, Kir currents were almost completely lost and the resting K^+ conductance was dramatically reduced. However, single cell RT-PCR still detected transcripts encoding subunits of the Kir2 family, suggesting that Kir4.1 is mainly responsible for Kir-mediated currents in astrocytes. This conclusion is also in line with our immunocytochemical findings since enhanced expression of Kir4.1 protein in GFAP-positive astrocytes accompanied the significant increase in Kir current amplitudes occurring during postnatal development.

Downregulation of astroglial Kir current has been found under pathological conditions, e.g. in human temporal lobe epilepsy (TLE). Hippocampal specimens were obtained from patients with pharmaco-resistant TLE, and astrocytes were characterized in the hippocampal CA1 region. Two forms of TLE were distinguished, Ammon's horn sclerosis (AHS) and lesion-associated epilepsy. Expression of Kir4.1 in human astrocytes was confirmed both on the mRNA and protein levels. Semiquantitative real time RT-PCR revealed a strong correlation between Kir current density and the amount of Kir4.1 mRNA in individual cells. The decrease in Kir current density due to downregulation of Kir4.1 in AHS patients will impair spatial K^+ buffering and suggests a role for astrocytes in seizure generation and/or spread in the sclerotic hippocampus.

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54 Alzheimer's disease. Beta-amyloid induces neuroinflammation via lipopolysaccharide receptor (CD14)

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Chronic neuroinflammation is considered to contribute to progressive neuronal injury in Alzheimer's disease. Here, we demonstrate that the key receptor in inflammatory defense against invading microorganisms, the LPS receptor (CD14), recognizes Alzheimer amyloid fibrils. Studies in microglial cells, CD14-transfected CHO cells and microglia derived from CD14-deficient mice show that CD14 mediates cellular activation provoked by amyloid peptide. Delineating its clinical relevance, we detected CD14 gene expression and CD14 protein immunoreactivity in brains of animal models of Alzheimer`s disease and in those of Alzheimer patients. Since only fibrillar but not non-fibrillar amyloid peptide was recognized by CD14, this interaction is based on physicochemical properties associated with fibrillar conformation of the peptide. CD14-

dependent cellular activation by amyloid peptide may represent a novel therapeutic target in Alzheimer`s disease.

Histochemical monitoring of apoptosis after cerebral ischemia and reperfusion in rat brain 55

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Intact adult rats received unilateral occlusions of the middle cerebral artery (MCAO) for 30- 60 or 90 min. Animals recovered from surgery within 2 days. 7 days after reperfusion, animals were killed by prolonged ether anesthesia and subjected to perfusion fixation with paraformaldehyde. Coronal brain sections were stained for markers of cellular damage. Immunoassays of brain homogenates were performed for quantitative assessment of cytochrome C and tissue transglutaminase (tTG). After 30 min of ischemia, rats exhibited only slight or no visible lesions of the cortex while animals of the 60- and 90 min groups showed extensive cortical lesions at the ischemic side of the respective brain hemisphere. A widespread distribution of DNA fragmentation and tTG immunostaining was observed in the periinfarct zone of the 30 min. group. In the 60 min group and more pronounced in the 90 min group, we found DNA fragmentation and immunostaining for tTG and cytochrome C also on the contralateral hemisphere, indicating degeneration through commissural pathways. Silver staining of brain sections of the 90 min group revealed the presence to agyrophilic plaques throughout the striatum, suggesting loss of neuronal perikarya in this region. Sham operated controls did not develop infarction. Animals that had been treated with vitamin E prior to MCAO and reperfusion showed significantly lower numbers of neurons stained for cellular damage markers. Our findings suggest that transient focal ischemia causes progredient apoptosis in brain regions ipsilateral and contralateral to the site of the lesion. Cytoprotective compounds like vitamin E may be capable of reducing this effect.

Neuronal glutathione supply by Müller cells during oxidative stress 56

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Glutathione plays an important role in the defense of cells against oxidative stress. Astrocytes, in co-culture with neurons, can support the neuronal glutathione metabolism by supplying glutathione precursors to neurons. We examined the metabolic glutathione support of Müller (glial) cells in acutely isolated mammalian retinae during oxidative stress.

Pieces of acutely isolated retinae from rats and guinea pigs were incubated for various time periods with different concentrations of hydroperoxide (H2O2) or tert-butylhydroperoxide (t-BHP). The thiol-reactive vital dye, CellTracker Green CMFDA, was used to detect intracellular levels of reduced glutathione (GSH). The cellular distribution of the fluorescent dye (corresponding to the GSH distribution) was visualized by means of a confocal laser scanning microscope. In control retinae, the GSH-sensitive-dye was exclusively detected in Müller cells, whereas the ganglion cells (GC) were devoid of fluorescence. After application of H2O2 or t-BHP to the retinal pieces, the GSH level in Müller cells decreased and the GC became fluorescent. This shift of fluorescence from Müller cells to GC depended on the duration and intensity of the oxidative stress. With increasing application time and concentration of the peroxides, the amount of intracellular GSH in Müller cells decreased and the GC became more and more fluorescent. This GSH shift from Müller cells to GC is not affected by an inhibition of the γ-glutamyltranspeptidase or by a block of the GSH synthesis with buthioninesulfoximine. Removal of the extracellular sodium ions during the peroxid application prevents the increase of the intracellular GSH in GC, but not the decrease of the intracellular GSH in Müller cells, therefore an involvement of a sodium-dependent uptake system is suggested. Furthermore, multidrug resistance associated proteins like MRPs are capable of transporting intact GSH molecules out of cells. We could detect MRP1, but not MRP2 mRNA in rat retinae by using the RT-PCR method. Immunohistochemistry revealed the expression of MRP1, but not of MRP2 proteins in Müller cells from rat and guinea pig retinae. During oxidative stress the GSH content in Müller cells was decreased, whereas in GC increased levels of GSH could be detected. Because this increase is not dependent on a de-novo synthesis of GSH, a transfer of intact glutathione (oxidized and/or reduced) molecules from Müller cells to GC is suggested.

57 Physiological properties of retinal Müller glial cells from the monkey *Macaca fascicularis* - comparison to human Müller cells

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Müller cells, the dominating macroglia of the retina, were demonstrated to be involved in pathological processes in the human eye (Bringmann et al., IOVS 40:3316-23, 1999). Similar effects were found in a rabbit animal model (Francke et al., IOVS 42:1072-9, 2001). However, the use of rabbits as a model for human eye diseases has some disadvantages arising from differences in the retinal anatomy, for example, the lack of blood vessels in most parts of the rabbit retina. Whereas the retinae of most other mammals possess a vascularization, a retina comprising a fovea is only found in primate species. Therefore, we performed patch-clamp and Ca^{2+} imaging experiments to characterize the physiological properties of Müller cells from a monkey, *Macaca fascicularis*, and com-

pared these data with those obtained from human Müller cells. Enzymatically isolated cells and retinal wholemounts were used.

Glial cells have been shown to express several ligand receptors and ion channels which had been thought to be neuron-specific. It is known from previous studies (Chao et al., J Neurocytol 26:439-54, 1997) that this expression is species-dependent. In monkey Müller cells we found dominant K^+ currents with inwardly rectifying and delayed rectifying properties. The membrane potential was close to the K^+ equilibrium potential (-88 ± 4 mV, n=20), the membrane resistance amounted to 44 ± 18 M Ω (n=20) similar to that recorded in rabbit cells (Francke et al., 2001). Whereas the membrane potentials were very similar in human Müller cells (-84 ± 7 mV, n=22), the mean value for the membrane resistance was significantly higher in human cells (179 ± 71 M Ω, n=22). Application of glutamate elicited transporter-mediated currents in human and monkey Müller cells. The $P2X_7$ agonist benzoyl-benzoyl-ATP caused inward currents of 151 ± 90 pA in human Müller cells (n=52) whereas only in a small percentage of monkey cells very small inward currents (about 20 pA) were recorded. Application of ATP evoked increases in intracellular Ca^{2+} in Müller cells in the human and the monkey retina. These effects are likely to be mediated by metabotropic P2Y receptors. Voltage-dependent Na^+ currents, Ca^{2+}-dependent K^+ currents and membrane currents mediated by $GABA_A$ receptors have been found in many human cells, but never in monkey Müller cells.

We conclude from these results that Müller cells from monkeys fail to display more similarities to human cells than cells from standard laboratory animals, and can not be recommended as better models for studies on glial cell features of the human retina.

Introductory Remarks to Symposium 7

Drug addiction: mechanisms and therapy

Volker Höllt

There is strong evidence that addictive drugs interact with an endogenous reward system in the brain. This system comprises a limbic circuitry of brain structures, such as amygdala, ventral tegmental area, nucleus accumbens, prefrontal cortex and other forebrain regions. Activation of these structures by addictive drugs or by other rewarding stimuli involves the modulation of the release of neurotransmitters, such as dopamine, glutamate, GABA, opioids and cannabinoids. Experimental interference with these neurotransmitter systems can alter the rewarding effects of addictive drugs. In addition, repeated administration of psychotropic drugs involves learning processes finally resulting in neuroplastic changes characterized as „addiction memory"". These adaptive processes are of long duration and may be responsible for the drug craving observed in long-term withdrawal/abstinence when all signs of somatic dependence have disappeared.

The present symposium will present molecular, behavioural and clinical aspects of drug addiction.

Neuroadaptive changes in gene expression in response to chronic opioid treatment will be addressed by V. Höllt. Using DNA microarrays the expression of about 8000 genes in the prefrontal cortex of rats chronically treated with morphine were analysed. Three persistently altered genes were found: *arc* and ania-3, proteins which are involved in synaptic function and *per*, a protein regulating circadian rhythmicity.

Cannabinoids and opioids are main neuromodulators of the reward system which have been proposed to act synergistically. Using mice with targeted deletions of the opioid peptide genes A. Zimmer will provide data which clearly show a close interaction of the cannabinoid and opioid system. Thus, cannabinoid withdrawal is attenuated in enkephalin knockout mice and conditioned place aversion of tetrahydrocannabinol is blocked in dynorphin-deficient animals.

Deprivation of alcohol in animals results in an enhanced ethanol consumption after reexposure to alcohol. Using this animal model for relapse and craving U. Schmitt will provide evidence that modulation of GABAergic neurotransmission clearly affects ethanol consumption and deprivation effects.

A detailed analysis of the glutamatergic mechanisms in addiction will be presented by W. Schmidt. Experimental evidence will be provided that interference with glutamatergic transmission alters the rewarding effect of addictive drugs. In addition, an increased reactivity of the glutamatergic system in long-term withdrawal is observed which appears to underly drug craving.

An overview of the reward system will be given by W. Hauber. By analysing the guidance of instrumental behaviour in rats he will provide evidence that signals related to expected natural reward are transmitted to the nucleus accumbens, a key region of the limbic cortico-striatal circuitry. The transmission of these signals in this structure involves NMDA- and AMPA glutamate receptors.

Clinically relevant neurobiological mechanisms of the development of drug addiction with an emphasis of the motivational aspects will be the topic of U. Havemann-Reinecke who will also provide an overview of the therapeutic stategies for dependence (substitution, anticraving drugs).

58 Gene expression profile in rat brain after chronic morphine treatment

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Opioid dependence is associated with adaptive changes in gene expression in brain. In the present study we used DNA microarrays (U34A; Affymetrix) to analyse the expression of about 8000 genes in the frontal brain of rats chronically treated with morphine and in rats after naloxone precipitated withdrawal. The frontal brain contains areas such as the frontal cortex, the preoptic area as well as regions supposed to be involved in addiction such as the prefronatal cortex and nucleus accumbens. Chronic treatment for 10 days with ascending doses of morphine (10 to 50 mg/kg bid) resulted in a more than twofold induction of 14 genes. The majority of these genes code for heat shock proteins (hsp70, hsp 27, hsp 40, hsp 105, GRP78 etc.). The expression of the heat shock genes was reversed in the morphine-treated animals after withdrawal precipitated by naloxone (10 mg/kg). The opioid antagonist, in turn, increased the expression of a set of genes which are predominantly transcription factors (krox20, CREM, NGFI-B, IkappaB etc). Few genes only remained increased after naloxone application. Such persistently changed genes code for arc, a cytoskeleton-associated protein which is induced by synaptic activity, ania-3, a splice variant of the Homer 1 protein which is critically involved in activity-dependent alterations of synaptic function and per2, a protein regulating circadian rhythmicity. For selected genes the changes in gene expression were confirmed by quantitative real time PCR and by *in situ* hybridization. These findings indicate that the persistent changes in long-lasting plasticity during opiate dependence do not primarily depend on the increased expression levels of neurotransmitter, receptor and/or ion channel proteins, but rather on altered pattern of synaptic connectivity.

59 Interactions between the opioid and cannabinoid systems.

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Recent studies have suggested a functional link between the endogenous opioid and cannabinoid systems. Cannabinoids and opioids act synergistically in the same neurobiological pathways to control several behavioral processes, including motivational responses, locomoter activity and nociception. We have used mice with targeted deletions of the enkephalin and dynorphin genes in order to investigate the potential role of these opioid peptides in mediating cannabinoid effects. Antinociception, induced in the tail-immersion test by acute THC, was reduced in dynorphin and enkephalin deficient mice, whereas no difference between genotypes were observed in the effects induced on body temperature, locomotion or ring catalepsy. These results indicate that the release of endogenous opioid peptides may contribute specifically to THC analgesia. Motivational responses to THC were evaluated in dynorphin knockout mice using a conditioned place preference test. THC has dysphoric properties in mice under most experimental conditions and produces a conditioned place aversion. Accordingly, Pdyn+/+ mice conditioned

with a dose of 5 mg/kg of THC spent significantly less time in the drug-paired compartment and obtained a lower place conditioning score. In contrast, dynorphin deficient mice injected with THC spent the same amount of time in the drug-paired as in the saline-paired compartment and showed a neutral place conditioning score. Thus, THC-induced conditioned place aversion was completely suppressed in mutant mice. During a chronic treatment with THC, the development of tolerance to the responses induced by this compound was similar in dynorphin knockout and wild type mice. Enkephalin deficient mice also developed tolerance, but significantly slower than wild type animals. Cannabinoid withdrawal syndrome, precipitated in mice that were chronically treated with THC by the injection of SR141716A, was significantly attenuated in enkephalin knockouts. In contrast, somatic withdrawal symptoms were only slightly reduced in dynorphin knockouts and not significantly different from wild type controls. Together our results indicate that the endogenous opioid system is involved in the regulation of specific THC effects, including antinociception, place aversion and physiological dependence.

Alcohol deprivation and the development of addiction 60

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The alcohol deprivation effect (ADE) has recently been introduced as a animal model for relapse and craving (Spanagel and Hölter, 1999). It uses ethanol treatment for several months. However, there is ample evidence that an ADE effect is observable already after 30 days of intermittent free-choice treatment. Thus it is of particular interest whether such an early effect mirrors motivational changes related to alcohol addiction. The present investigation verified the alcohol deprivation effect in a free-choice setup and studied effects of drugs interacting with the GABAergic system on this motivational change in alcohol consumption.

A total of 50 male PVG/OlaHsd rats was analyzed for free choice ethanol drinking behavior without and with pre-exposure to drugs acting on the GABAergic system in different manners either by facilitating or inhibiting transmission. For pre-treatment, animals received the benzodiazepine-site agonists or antagonists diazepam, flumazenil or Ro15-4513, or the GABA uptake inhibitor tiagabine via the drinking water for 4 weeks (day -21 until day 7). On day 0, two bottles containing 5 and 12 % ethanol were added. On day 7, GABAergic drug exposure was discontinued and drug solutions replaced by water. Between days 8 and 35, three alcohol deprivation periods of one to three days were randomly implemented.

The animals ingested substantial amounts of ethanol which was differentially affected by the GABAergic drugs. Diazepam increased whereas flumazenil decreased ethanol consumption significantly by about 30%. Without GABAergic pre-treatment, a significant alcohol deprivation effect indicated by enhanced ethanol consumption after re-exposure to alcohol was observed after the third deprivation phase. This kind of deprivation effect was prevented by pre-treatment with diazepam or flumazenil, it was unaffected by Ro15-4513 and advanced by tiagabine.

These findings indicate that modulation of GABAergic neurotransmission affects subsequent ethanol consumption and deprivation effects. Since enhancing of the GABAergic tone by the GABA uptake inhibitor tiagabine or by the benzodiazepine diazepam had different behavioral consequences it seemed likely that the two drugs induce differential adaptive changes leading to distinct alterations in the motivation to consume alcohol depicted by the early alcohol deprivation effect.

Spanagel, R. and Hölter, S. (1999) Alcohol & Alcoholism 34:231-243.

61 Glutamatergic Mechanisms in Addiction

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At least two brain mechanisms are of particular relevance for addiction, these are the brain reward system and the learning system. The brain reward system mediates pleasure; the learning systems built up a drug-memory and context-memories. The main neurotransmitters of the reward system are dopamine, endogenous opioids, endogenous cannabinoids and glutamate. Glutamatergic pathways from the prefrontal cortex to the midbrain dopamine neurons and those projecting to the nucleus accumbens are integral parts of the reward system and experimental interference with either of these glutamatergic pathways, and with either of the different glutamate receptors, consistently alters the rewarding effect of addictive drugs.

The development of addictive behaviour includes learning processes in which glutamate is crucially involved. However, the nature of the underlying learning processes, as well as the role that glutamate plays, are still enigmatic. It is proposed here that sensitization represents one of these learning processes since all addictive drugs produce sensitization. Sensitization refers to the intensification of a behaviour upon repeated administration of a drug. Two different forms of sensitization exist, a context-independent form, that may result from an increased cellular effect of a drug, and a context-dependent (associative) form of sensitization which results from drug-induced changes in complex neuronal circuits. In the latter case, the sensitized response is partly due to the drug and partly, or completely, due to the context. Mostly, a stimulus or the environment represents the context, but also an internal state, or even a drug-induced state, can act as a context. Context-dependent sensitization is built up gradually and is very persistent over time. It can be considered as a form of habit learning and is supposed to take place in the basal ganglia.

Acute withdrawal from addictive drugs produces a compensatory hypo-dopaminergic state, associated with depression- and anxiety-like mood. Even over long withdrawal phases, the memories for drug-associated stimuli do not disappear, in contrast, stimulus-induced reinstatement of drug seeking in rats, which may represent a model for human relapse, progressively increases over the first two months of withdrawal; this is called the incubation effect. Further, during long-term withdrawal, and after disappearance of all signs of somatic dependence, an increased reactivity of the glutamatergic system to

drug-associated stimuli has been shown. It is tempting to speculate that the glutamatergic hyper-reactivity underlies craving. In this case, anti-glutamateric drugs could be taken into consideration as potential anticraving drugs.

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Control of Behaviour by Reward-Related Stimuli 62

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Natural rewards such as food act on the limbic cortico-striatal circuitry, a neural network comprising the amygdala, prefrontal cortex, nucleus accumbens, ventral tegmental area and other forebrain regions. This pathway is not only involved in processing of natural rewards and in neural plasticity underlying reward-related learning, but also in processing of rewarding effects of addictive drugs. A major mechanism underlying drug addiction might involve aberrant associative learning processes mediated by the limbic cortico-striatal circuitry through which stimuli associated with drug taking elicit drug seeking and reinforce instrumental responses to obtain a drug. The experiments presented here examined neurochemical signals in the limbic cortico-striatal circuitry involved in control of reward-directed behaviour by incentive stimuli. In particular, we investigated the role of stimulus-induced reward expectancy which is an important factor of guidance in reward-directed behaviour. We used natural instead of drug rewards, however, many neuronal adaptions in animal models of addiction are very similar to those implicated in models of learning and memory using natural rewards. Therefore a better understanding of associative learning processes in natural brain reward systems might enhance understanding of respective brain mechansims underlying drug addiction. The core compartment of the nucleus accumbens (ACB) is a key region of the limbic cortico-striatal circuitry through which information on the motivational significance of stimuli influences the selection and execution of reward-directed instrumental responses. We investigated the role of the ACB in a lever release task in which the speed, i.e. the reaction times (RT), of instrumental responses were guided by the expected food reward magnitude (1 vs. 5 food pellets) signaled in advance by discriminative stimuli. In rats which acquired this task, RT of instrumental responses was significantly shorter to the discriminative stimulus predictive of high reward magnitude. In these animals, blockade of AMPA/KA or NMDA receptors in the ACB produced a general increase of RT, but left RT determination by stimulus-associated reward magnitudes unaffected. Similar effects were observed after a blockade of intra-ACB dopamine receptors. Furthermore, blockade of AMPA/KA or NMDA receptors during acquisition of the task did not impair guidance of instrumental responding by stimulus-associated reward magnitudes, but produced a general increase of RT. Together, these data reveal that the ACB is not required for the knowledge of the contingencies between incentive stimuli, instrumental actions and their outcomes. However, these findings suggest that stimulation of NMDA and AMPA/KA receptors in the ACB is critically involved in guiding the intensity of instrumental responses to stimuli predictive of reward.Taken together, the data provide further support to the notion that signals related to expected natural reward are transmitted to the ACB by glutamatergic projections from cortical and limbic regions invol-

ved in processing of the motivational significance of stimuli, such as the basolateral amygdala and the prefrontal cortex. Given that transmission of signals related to expected drug reward also involves intra-ACB NMDA- and AMPA receptors, this raises the possibility that drugs aimed at these receptors might be of use in the treatment of drug craving which is elicited by drug-associated stimuli. Supported by the DFG.

63 Ecstasy, dependence and pharmacotherapy

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Alcohol and other drug induced experience of ecstasy with euphoria as well as drug reinforcing-, sensitivation- and conditioning- mechanisms are described to be important for the development and maintenance of psychic dependence and specially changes of dopaminergic neuronal activities seem to be critically involved. By influencing these various mechanisms dopamine basically change the motivation of an individual towards addictive drugs leading to intensive drug seeking behaviour, which is clinically difficult to treat. Pronounced individual differences are described. Experiences of ecstasy could be one important factor, but are not necessary for. The occurrence of psychic dependence with drug seeking behaviour is the outcome of a number of variables including genic, neuronal (dopamine and interactive transmitters f.e glutamate, GABA, serotonine, opioid peptides), kind of drug, developmental, age, neurodegenerative, comorbidity of other psychiatric diseases and environmental elements that interact to produce profound individual differences in both, initial and longterm responsivness to addictive drugs. Single representive results from animal and clinical studies especially on (genetic) individual differences will be presented to support this statement. Basing on the neurobiological mechanisms of addiction different clinical (pharmaco)treatment strategies (detoxification, substitution/maintenance, anti-craving) will be discussed.

Introductory Remarks to Symposium 8

Precise timing in the brain: linking neuronal activity and behavior

Detlef Heck and Fahad Sultan

Over the past decade evidence has accumulated that precisely timed neuronal activity in the neocortex is associated with various aspects of behavior. The role of these precisely timed activity patterns in the neocortex in the control of motor output is still unclear. Since synchronization occurs between distant parts of the neocortex, even bridging hemisphere boundaries, it is possible that synchronized activity is also used in the communication between different brain structures involved in the control of movement. The symposium shall bring together ideas about spatio-temporal activity patterns in neocortical neural networks with ideas about the function of the cerebellum and finally the neuronal control of precisely timed motor output.

Moshe Abeles has proposed that neocortical activity is organized in form of chains of synchronously active groups of neurons („synfire chains"). He later showed experimentally that precisely timed spike patterns – presumably reflecting a traveling „synfire chain" – occur during behavioral tasks in awake behaving monkeys.

Stefan Rotter and colleagues investigate and compare the contribution of single neurons and large populations of those to the control of various parameters of movement. They report that, when reconstructing movement trajectories on a single trial basis, the relevant time base of the underlying neuronal activity measures in tens of milliseconds rather than milliseconds.

Jeff Keating has used multiple electrode recordings in sensorimotor cortex to record activity during a reaching-grasping movement. He investigated the temporal correlation of neuronal activity and could show that successful and failure trials had identical changes in spike rate but were separable based on the dynamics of the correlation of neuronal activity prior to the grasp.

Peter Thier has shown using recordings from Purkinje cells in monkeys trained to do saccades of different amplitudes, that the population activity gives a precise temporal signature of saccade onset and offset. Further results suggest that the population response can be modified by changing the weights of the contribution of individual Purkinje cells, thus resulting in a change of saccade amplitude.

A cerebellar role in the precise timing of ball release in overarm throwing movements has been suggested by the ground breaking work of Jonathan Hore. His findings suggest that the combination of finger and hand muscle activity has to be controlled with millisecond precision in order to produce the desired motor output.

The cerebellum potentially detects precisely timed spike pattern generated by the neocortex and, triggered by their occurrence, produces the associated output. Precisely timed spike patterns may thus be a key to neocortical-cerebellar interaction (V. Braitenberg). Since the cerebellum is mostly involved in motor control we will try to build a bridge to temporally precise motor output.

64 Scales for Computational Elements in the Cortex

Moshe Abeles

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The classical parcellation of cortex is based on cyto- (myelo-) architectonics. Each area is expected to have a distinct function. This is a fundamental parcellation of function till our days. The same notion is taken also to mean that activity is induced by inputs to each area, the input information is processed for a while in the area and then outputted to another area.

In the last few decades the regional nature of function was refined by further parcellation of cytoarchitectonic areas into patches (Columns, Blobs, Barrels) with unified function. Within each patch the neurons share some specific common function (orientation, whisker). The idea of input—processing—output holds as well for the patchy cortical architecture

Most neurophysiological studies of single neurons in the cortex assume that each neuron has a distinct function. Experiments in which repetitive stimuli of the same kind are given for many times (or the same motor action is executed over and over again) support this notion. However adjacent neurons may have very different functions. This is stressed by the fact that cross correlations between spike trains of next neighbors is often flat or very week. When recording in association cortices while an animal behaves along a fixed behavioral paradigm one find that approximately one third of the neurons show "task related activity". But even this fraction may typically be further fractionated among different roles (epochs) within the task. Thus the special dimension of unity of function may be reduced to a single neuron.

However, in behaving animals, with paradigms that allow diversity one often observes periods in which the same neuron takes part in different functions. Such periods may last 0.1-1 sec. This has been shown by our group for the prefrontal and posterior parietal cortices long ago, and recently for the motor cortex. Such functional time-multiplexing is in accordance with the findings that cross-correlations between a pair of cells are dynamically changing on a time scale of a fraction of a second.

Extrapolating from the above one may ask is it possible that every spike participates in a different function?

On the theoretical ground this is quite possible. Synfire chains clearly posses the ability to multiplex functions on a spike-by-spike basis, and to readout such multiplexed processes without confusion. Experimental results on precise firing sequences show that it is possible to sort-out spikes which are part of a given process from spikes which are not.

The above will be discussed with examples from our experimental results using multi-electrode recordings in behaving monkeys.

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Decoding Neuronal Population Activity Associated with Arm Movements

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We measured the trial-by-trial variability of neuronal activity in primate motor cortex during repeated arm movements and characterized its sources (Nawrot et al. 2000). We found that the variability in simultaneously recorded multiple single-neurons spanned a wider range than reported in visual cortex, with a significant fraction of neuron pairs co-varying their firing rates across trials. Variability changed dynamically in a task-related manner, being considerably lower during movement execution than during preparation, and even more so than during waiting. A systematic evaluation of spike count variability vs. spike time irregularity enabled us to identify different sources of variability, operating on different time scales: rapid changes in network activity, related to the task, and slow changes, not related to the task. The latter explains more than one half of the trial-by-trial variability in motor cortex. These different ongoing dynamics could play an important role in motor cortical function, and must be taken into account when trying to read the neural code. A time-resolved variant of the classical concept of 'directional tuning' was used to analyse the degree to which the direction of the arm movement can be inferred from the single-trial responses of neuronal populations, despite their high variability (Rickert et al. 2000). In particular, we tested feasibility and robustness of early prediction of arm movement direction from single-trial spike trains recorded in monkey primary motor cortex during movement preparation and execution. We found that neuronal tuning profiles during an instructed delay were modulated in time, in a task-related fashion, and always depended on prior information about the required movement. Time-resolved single-trial activity of small random populations of 50-100 neurons was sufficient to reliably predict movement direction throughout the task, some 150 ms after cueing and up to one second before its execution. Prediction was essentially error-free, bounded only by the amount of prior information available to the monkey. To probe the spatial scale of neuronal encoding of movements, we explored to which degree local field potentials (LFP) recorded from the motor cortex can serve as an alternative signal to spike trains of well-isolated single-units. Monkeys performed center-out arm movements to one out of eight directions, while multiple single-unit activity and local field potentials were recorded from the motor cortex (Cardoso de Oliveira et al. 2001). We found that a single LFP channel carried essentially the same amount of information about the direction of an upcoming movement as the spike train of a single cell (Mehring et al. 2003a,b). This also demonstrates the feasibility of using LFPs for the prediction of voluntary arm movements, e.g. for the control of neuronal motor prostheses.

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66 Directional information flow in sensorimotor cortex during reaching as revealed by the gravitational transformation

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A linear array of microwires was placed in rat sensorimotor cortex and multiunit activity recorded during reaching. All reaches were videotaped and accurately targeted reaches were sorted into success and error trials. Waveform data was continuously recorded, noise removed (Gerstein et al, 2002), and spikes detected using a three-point template. Spike times were analyzed using the gravitational transformation. Trials were aligned on the detection (by a proximity switch) of the rat's paw near the pellet. The data were analyzed in partially overlapping 50ms pieces during the trial. Each 50 ms piece was examined for synaptic delays of -20 to +20 ms using 1 ms resolution kernels in separate gravity runs. The gravitational method transforms synaptic delays of each spike train into corresponding particle movement. The total distance each particle moved under each condition was color-coded and plotted on a grid (of synaptic delay vs trial time). Movement significance was confirmed using a shuffle predictor. Electrodes showing arm-related spike discharge tended to fire synchronously throughout the trial. In addition, between 200 and 0 ms before pellet contact, the more medially located electrodes recorded spikes that tended to fire 4-6ms before the more laterally located electrodes. This latter relationship occurred during trials where the rat successfully grasped the pellet; such uni-directional flow was not seen during trials when an error was made. During this same period, there was no difference in rate between the success and error trials. Examination of the spike times showed that the particle movement was due to an increase in near-synchronous activity on the other electrodes. Autocorrelations revealed no tendency for oscillation. The data reveal a unidirectional flow of information from motor to sensory cortex occurring before pellet contact when the animal will successfully grasp the pellet. This discrepancy between success and error trials may reflect the importance of an efference copy of the motor plan being sent to sensory cortex.

67 Encoding of movement time by populations of cerebellar Purkinje cells

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One of the earliest computational principles attributed to the cerebellum has been the measurement of time. This idea was originally suggested on purely anatomical grounds

and taken up again much later to explain some of the motor and perceptual deficits observed in cerebellar patients. The contribution of the cerebellum to eye movements, on the other hand, has traditionally been discussed in the context of motor learning. This view has recently received support from the loss of saccade adaptation, one of the key examples of motor learning, following lesions of the posterior cerebellar vermis (Barash et al., 1999, J Neurosci 19: 10931-39). However, the relationship between the properties of saccade-related vermal Purkinje-cells (PCs) and the behavioural deficits has as yet withstood clarification.

In order to reconcile the seemingly unrelated concepts of timing and motor learning we trained monkeys on smooth-pursuit eye movements and saccades and recorded vermal (lobuli VIc and VIIA) saccade-related Purkinje cells. Many Purkinje cells in this area show pursuit-related responses, saccade-related responses or combinations of the two and usually lack responses to the presentation of visual targets, guiding the oculomotor behavior. The saccade-related responses are usually directionally-selective and show preferences for saccade amplitude or duration which differ widely between cells. However, unlike individual Purkinje cells, which fail to provide a reliable estimate of saccade amplitude or duration, the population response of a large group of PCs gives a very precise temporal signature of the saccadic eye movement: the population burst starts about 65ms before saccade onset and peaks precisely at saccade onset, independent of saccade duration. Finally, the end of the population burst corresponds very precisely with the end of the saccade.

We hypothesize that the population burst helps to determine the end of the saccadic eye movement and, furthermore, that changes in the duration of the population response might be the basis of saccadic adaptation. Modifying the time course of the population response by changing the weights of the contributing individual PCs, discharging at different times relative to the saccade, would directly translate into changes in saccade amplitude. Obviously, this scheme, which tries to lead saccadic learning back to an optimization of a representation of time, is not confined to the saccadic system. Rather, its virtue arises from the fact that it might be applicable to any motor or non-motor function dependent on the availability of a precise representation of time.

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Precision and timing of motor output 68

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Precision in the timing of ball release was studied in overarm throws made by skilled, unskilled and cerebellar subjects. All subjects were instructed to throw accurately at different speeds; arm kinematics were recorded with the search-coil technique at 1000 Hz. The results show that finger opening is produced by central commands and not by back force from the ball on the fingers or by proprioceptive feedback. In a series of 30 throws the best skilled subjects had a timing window for finger opening and ball release of < 5 ms when measured with respect to different arm kinematic parameters. In contrast, unskilled subjects and cerebellar patients did not time finger opening precisely, and consequently, did not hit the target accurately. Insight into the central programming

of finger opening in skilled subjects came from the finding that plots of hand angular position in space against finger opening (angular position) were similar for throws of different speeds. That is, for these two parameters a fast throw was the same as a slow throw speeded-up. Following the ideas of Thach, one interpretation of these results is that precise timing of finger opening results from a role of the cerebellum in combining motions of different body parts (finger and hand) into a single organized movement. In this case, precision in the timing of ball release in throwing would result from accuracy of the cerebellum in combining motions of finger and hand, rather than from accuracy in triggering finger opening at the exact time.

69 Spatio-temporal Activity Patterns as a Key to Cerebellar Function

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In no other part of the nervous system is the internal connectivity as thoroughly known as in the cerebellar cortex. Moreover, although other cortices are not known in comparable detail, one can confidently assert that the pattern in the cerebellum is unique.

This would seem to make it possible to go directly from the elementary mesh of the cerebellar network to a definition of its global operation, and hence to an explanation of the "functions" of the normal cerebellum and of the "symptoms"of its derailment, as they appear to the clinical neurologist. Nobody has succeded in building this bridge, in spite of some proposals which were seductive in their generality, but too general to serve as an explanation of the uniqueness of the cerebellum.

The stagnation of our theorizing is not caused by lack of experimental findings, which have been forthcoming at an impressive rate in recent years. Rather, it seems that most of the experiments were not so much aimed at an elucidation of the special kind of computation typical for the cerebellum, as at questions which apply to the nervous system everywhere, such as membrane physiology and plasticity on one hand, the mapping of input and output connections on the other.

In this situation it seems legitimate to take a fresh start by reproposing once more the level of analysis where the cerebellum is most characteristically itself, the level intermediate between cytology and fiber bundle tracing, that of the geometry of the intracortical fiber felt.

What is "simultaneous"? Tactile coactivation in human subjects reveals requirement for millisecond precision for induction of plastic changes

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The importance of temporally correlated inputs have been hypothesized to play a key role in mediating plastic changes. In order to test directly the timing requirements for the induction of plastic changes, we used a perceptual learning protocol consisting of tactile coactivation: Synchronous neural activity was generated by simultaneous pairing of tactile stimuli (Godde et al. 1996, 2000). Coactivation applied to the tip of the right indexfinger (IF) lowered spatial discrimination thresholds, while performance of the left IF remained unaffected. Combined assessment of discrimination thresholds and recording of somatosensory evoked potentials in human subjects revealed that the gain of performance was correlated with the amount of cortical reorganization (Pleger et al. PNAS 2001).

Here we report experiments designed to explore the "window of simultaneity" required to evoke perceptual learning. We tested 20 right-handed subjects in a 2-alternative-forced-choice simultaneous spatial 2-point discrimination task. To apply coactivation, a small device consisting of two stimulators that were independently controlled (diameter of stimulation tips 0.5 mm, separation 5 mm), was mounted to the tip of the right index finger.

Introducing a constant delay of 50 ms between the paired stimuli eliminated completely the improvement of discrimination performance observed for zero delay (full simultaneity). Using 25 ms delays restored the coactivation-induced gain in discrimination, yet subjects did not reach the full level of improvement. 35 ms delays revealed an intermediate efficacy of coactivation. Controls based on single-point stimulation had no effects confirming the need for co-activation. The results indicate that global, perceptual learning processes are under the control of timing in the few millisecond range. Such a fine-scale timing control has so far only been demonstrated to be effective at a cellular level.

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Godde B. Stauffenberg B. Spengler F & Dinse HR (2000) J Neurosci 20: 1597-1604
Pleger B. Dinse HR. Ragert P. Schwenkreis P. Malin JP & Tegenthoff M (2001) Proc. Natl. Acad. Sci. USA 98, 12255-12260
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Effects of timing: Switching cortical map reorganization and perceptual learning

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Temporal patterns of sensory inputs appear to determine the organization of functional receptive field properties and the topography in cortical representations. A number of

former studies in humans and animals have shown that tactile two-point stimulation (coactivation) leads to an enlargement in primary somatosensory cortex (SI)[1-3].

In order to test the effect of timing, we compared a protocol of synchronous (temporal correlated stimulation) and asynchronous (temporal uncorrelated) tactile two-point stimulation to evoke cortical plastic changes in SI of rats and humans. The stimuli consisted of two independently generated Poisson processes (mean ISI 970 ms). We asked in how far the reorganizational changes induced by both protocols differed on a cortical and a psychophysical level. In rats, we measured reorganizational processes induced by coactivation, in which two skin sites of the hindpaw were activated synchronously or asynchronously. Extracellular recordings of multi-unit activity were performed under Urethane anesthesia. We used two approaches. First we specified the size of receptive fields (RFs) on the hindpaw and their corresponding cortical representations of the stimulated skin field by "handplotting" before (pre) and after (post) synchronous and asynchronous coactivation over 6 hours. In a second experimental setup we used the "response-plane"-technique, to gain information about the 2-dimensional RF-structure. For both approaches, we found that synchronous 2-point stimulation resulted in a fusion of the cortical representations of the two activated skin sites, whereas an asynchronous protocol induced a separation of the representations in the cortical maps consisting in a repulsion of the respective skin sites.

As an indirect psychophysical marker, we determined the 2-point discrimination thresholds of the right indexfinger of human subjects. We tested 20 right-handed subjects in a 2-alternative-forced-choice simultaneous spatial 2-point discrimination task[1-3]. To apply coactivation, a small device consisting of two stimulators that were independently controlled (diameter of stimulation tips 0,5 mm, separation 5 mm), was mounted to the tip of the right index finger.

We confirmed that a synchronous coactivation (using one Poisson process) for 3 h lowered the spatial 2-point discrimination acuity. In contrast, asynchronous coactivation (using two Poisson processes; same statistics and average frequency) impaired discrimination performance, as indicated by an increase of discrimination threshold in human subjects. Control experiments using stimulation of only one single skin site had no effect.

These results indicate that manipulating the nature of correlated activity drives complementary forms of cortical map reorganization in rats, and leads to complementary forms of perceptual learning in humans.

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[2]Godde B, Stauffenberg B, Spengler F, Dinse HR (2000) J Neurosci 20:1597-1604
[3]Pleger B, Dinse HR, Ragert P, Schwenkreis P, Malin J, Tegenthoff M (2001) Proc Natl Acad Sci USA 98:12255-12260

Electrical Source Activity and Interregional Coherences of the Human Brain During Visuomotor Tasks

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Imaging studies in humans suggest that visuomotor control of forelimb and eye movements involves reciprocal connections between striate, extrastriate, parietal, motor and frontal areas related to movement performance and visuospatial coding of movement direction. Coherent electrical brain activity has been demonstrated to be associated with perceptual events in mammals. The aim of our study was to investigate the functional role of the human extrastriate visual area V5 in controlling visually guided hand movements by EEG recordings with subsequent reconstruction of sources and the patterns of coherence in the EEG between extrastriate visual areas, parietal and motor cortex. Eleven subjects performed visually guided right hand movements, either tracking a horizontally moving target or performing a center out task to a stationary target by moving a cursor using a joystick. We used four conditions: tracking with central fixation and center out with central fixation, each of them accompanied by a replay condition without hand motor activity as a control task. Continuous EEG with 96 electrodes was recorded. EEG signals were digitally filtered (1-60 Hz) and the location and time course of source activity were analyzed with the EASI source reconstruction program. Interregional coherence of EEG signals in different frequency bands was also analyzed.

Our results showed strong bilateral source activations in area V5 during visually guided hand tracking and reaching movements after the replay condition is subtracted and demonstrated a neural network involving left sensorimotor cortex, bilateral posterior parietal cortex and SMA . The time course of activity for V5 could be grouped in two components, an early one, ranging from 125 ms to 295 ms and a late component from 771 to 884 ms. We found coherences between both parietal cortices in all four conditions. Coherence between V5 and parietal cortex or motor cortex was not found in any condition in either frequency range.

In conclusion visual monitoring during tracking and reaching activates area V5 in a large-scale sensorimotor network. Coherences among parietal cortex mean global space representation and coordinate transformations. Coherence between V5 and motor cortex was not found which agrees with our results from animal experiments and could mean that areas have to be more directly connected to be forced into coherence.

73 RTMS elicits tactile discrimination improvement and parallel plastic reorganization in human SI

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rTMS (repetitive transcranial magnetic stimulation) is widely used to investigate different aspects of cortical excitability and inhibition, which play an important role in controlling plasticity. Here we demonstrate, using psychophysical testing and parallel functional magnetic resonance imaging (fMRI), that subliminal rTMS applied with a figure-eight coil positioned over the digit representation of left somatosensory cortex (SI) evokes plastic changes on the stimulated, ipsilateral cortical hemisphere. Two rTMS sessions (5 Hz, 25 trains with 50 single pulses, for 10 min) with a rest period of 1 hour between sessions resulted in selective and reversible reorganization of cortical finger areas in SI. As an indirect marker of cortical reorganization we measured tactile spatial two-point discrimination of the right index (IF) and ring finger (d4) in an AFC task before and after rTMS. The left IF served as control. rTMS revealed a significant lowering of thresholds for the right IF and d4, which was reversible within 135 min for the IF and within 90 min for d4. The left IF was not affected by rTMS. A combined assessment of discrimination thresholds and fMRI recordings revealed a rTMS-induced enlargement of SI, which was linearly correlated with the individual gain of discrimination improvement after rTMS. The results indicate that a stimulation protocol resembling those used in LTP studies, applied from outside directly to selected brain regions can induce meaningful cortical reorganizations paralleled by improvement of discrimination thresholds.

Additionally we compared our findings with a previous study, where we applied a peripheral stimulation protocol (coactivation) to the tip of the right IF for a mean period of 3 hours (mean stimulation intensity 1 Hz) in order to induce short-term plasticity within the somatosensory cortex (Pleger et al. 2003, this meeting). As described for the rTMS induced discrimination improvement, the coactivation protocol also results in a similar lowering of spatial discrimination thresholds. We therefore compared both approaches (5Hz rTMS/ coactivation) on a cortical level using fMRI measurements before and after the application of both protocols. We found that a peripheral coactivation of receptive fields on the fingertip resulted in reorganizational processes in primary as well as secondary somatosensory cortex. In contrast, 5 Hz rTMS applied directly to primary somatosensory cortex lead only to an enlargement of SI, indicating a different way of information processing despite the same outcome observed psychophysically.

Functional magnetic resonance imaging of the human brain: Cortical reorganization controls somatosensory short-term learning. 74

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In this study we investigated the influence of tactile coactivation-induced changes in cortical somatosensory representation. We therefore performed fMRI with electrical stimulation of both index fingers. 10 right-handed subjects were tested in 2-alternative-forced-choice simultaneous spatial 2-point discrimination task of both index fingers before and immediately after tactile coactivation was applied only to the tip of the right index finger via a small solenoid over a period of three hours. For coactivation procedure we chose a randomized tactile stimulus to induce cortical reorganization. The underlying idea was to perform a simultaneous stimulation of adjacent cutaneous receptive fields on the finger tip.

BOLD (blood oxygen-level-dependent) responses of all fMRI sessions could be assigned to contralateral primary (SI) and secondary somatosensory cortex (SII), as well as SII of the ipsilateral side. After coactivation subjects showed powerful lowered two-point discrimination thresholds of the coactivated finger only. SPM99 random effect analysis revealed a coactivation-induced significant increased response of both contralateral somatosensory representational areas (SI, SII) indicating a parallel enlargement of the representation of the right index finger. Individual side-corrected difference maps (post - pre) revealed a linear correlation between spatial discrimination improvement and increased SI response. Conceivably, plastic processes in SI were scaled with the degree of the individual perceptual improvement. Less fine-grained somatotopic as well as missing separate finger representation in SII might explain the missing relation to overall discrimination performance. Our findings indicate a powerful remodelling effect within SI and SII induced by tactile coactivation predicting discrimination improvement. fMRI seems therefore to be a suitable method not only in investigating somatotopic organization, but also in uncovering short-term learning dependent reorganization in human somatosensory cortex.

Temporal Pattern Recognition Based on Instantaneous Discharge Rate Coding in a Simple Auditory System 75

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Auditory pattern recognition by the CNS is fundamental to acoustic communication. For the neural processing of amplitude modulated sounds the instantaneous spike rate rather

than the mean neural activity may be the appropriate coding principle. We compared both coding parameters in a simple auditory system. Since crickets communicate with stereotyped patterns of constant frequency syllables, they are established models to investigate the neuronal mechanisms of auditory pattern recognition. We demonstrate that the afferents are not tuned to syllable patterns of certain repetition rates but they act as syllable onset detectors, which preferentially respond to the beginning of sound pulses. This explains why the duration of syllables is not relevant for phonotactic behaviour. When stimulated with different temporal sound patterns the response of a thoracic low-order interneuron is very similar when the average discharge rate is considered. However, analysing the instantaneous discharge rate, demonstrates that the neuron responds with prominent peaks in its instantaneous discharge rate to syllable rates close to the species-specific sound pattern. The occurrence and repetition rate of these peaks in the neuronal instantaneous discharge are sufficient to explain temporal filtering in the cricket auditory pathway since they closely match the tuning of phonotactic behaviour to different sound patterns. Temporal filtering or "pattern recognition" does not happen as previously proposed in the brain but occurs at the earliest stage in the auditory pathway. For the first time our data emphasize the importance of instantaneous discharge rate coding in auditory processing rather than integration of neural activity over long time periods.

76 Investigating cortical network dynamics with combined intracellular and multi-electrode extracellular recordings

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Neurons communicate with each other and interact with the surrounding network by synaptic transmission of spike activity. These synaptic interactions are not static but change depending on a variety of parameters, such as the history of a synapse's activity (e.g. [1;2]) and the dynamics of the ongoing network activity [3;4]. The investigation of neuronal interaction in cortical networks has been carried out using correlation analysis of spike trains recorded *in vivo* or through direct measurement of synaptic transmission by dual intracellular recordings *in vitro*. Both approaches, however, are deficient for different reasons: The correlation analysis of spike trains provides important insights allowing to examine dynamic changes in the interaction between neurons depending on the network activity [3;4]. But, because only spike times are measured, information about the cellular mechanisms underlying these dynamic changes are not accessible. Conversely, measurements in *in vitro* preparations have technical advantages that allow direct intracellular measurement of a single synapse's currents and potentials [5]. But, because of the absence of ongoing network activity, *in vitro* preparations constitute an unsuitable environment for the investigation of neuronal communication under *in vivo* conditions. In previous work we have investigated the effects of background activity on neuronal interaction in rat neocortex *in vivo*. We have shown that the summation of synaptic input in the neocortex *in vivo* is linear and independent of the level of network activity. But, we could also show that increased network activity critically reduces both amplitude and duration of EPSPs [6;7]. Consequently, because of the reduced EPSP-amplitude the effect of population synaptic inputs on a neuron's spike response probability depends on the level of background activity. Functionally even more relevant are the effects of changes

in network activity in the time domain. Due to the shortening of EPSP duration with increased network activity, the time window for summation of synaptic inputs is drastically shortened. This should critically affect whatever mechanism is responsible for the generation and propagation of temporally precise spike activity (e.g. [8;9]).

Here, we have combined multi-site extracellular recording with single intracellular recordings *in vivo* to simultaneously monitor neocortical network activity and its effect on subthreshold membrane potentials. We have used these data to investigate spatio-temporal aspects of neuronal interaction in a spontaneously active network.

*D.S. and F.K. contributed equally.

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Detection of sequences in the cerebellar cortex: Numerical estimate of the possible number of sequences represented

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The two major cortices of the brain - the cerebral and cerebellar cortex - are massively connected through intercalated nuclei (pontine, cerebellar and thalamic nuclei). We suggest that the two cortices co-operate by generating precise temporal patterns in the cerebral cortex which are detected in the cerebellar cortex as temporal patterns assembled spatially in the mossy fibers. We will begin our review by showing that the tidal-wave mechanism works in the cerebellar cortex as a read-out mechanism for such spatio-temporal patterns due to the synchronous activity which they generate in the Purkinje cells - the output neurons of the cerebellar cortex. We will further review the anatomy of the mossy fibers and show that within a “beam”, or “row” of cerebellar cortex the mossy fibers in principle could embed a vast number of tidal-wave generating sequences. Our analysis shows that the number of possible sequences is critically dependent on several anatomical parameters, some of which have not been explored in great detail yet. Depending on the anatomical parameters our estimates range from a theoretical number of 10^71 to up to 10^343 for one “row” of the cerebellum of a mouse. Thus the cerebellar mossy fiber-granule cell-Purkinje cell system can potentially detect/ store an enormous number of temporal patterns.

78 Simulating the cerebellar tidal-wave – variability in axonal conduction velocity constrains noisy inputs

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The tidal-wave mechanism as a model of how the cerebellar cortex detects precise spatiotemporal patterns depends critically on the parallel fiber conduction velocity. Here we examined through numerical simulations whether the experimentally observed variability in axonal conduction velocity renders the tidal-wave implausible. In addition, our simulations tested how noise in the tidal-wave input sequences affects its summation in the molecular layer. Our results show that the tidal-wave mechanism is stable despite the variability in the parallel fiber conduction velocity. Our noise analysis also shows that a temporal jitter in the input with a standard deviation $\sigma_j = 0.6$ ms can be tolerated. Our simulations further revealed that an input jitter of up to $\sigma_j = 1.2$ ms can be handled, given that the number of inputs is sufficiently high (around 100). With even higher numbers of input lines (500), the input jitter can even be larger. Consequently, the exceptionally large convergence of parallel fibers on Purkinje cells might reflect the adaptation of the system to deal with a reasonable large amount of jitter in the granule layer input.

Introductory remarks to Symposium 9

Ontogenetic cell death in the nervous system

Kerstin Krieglstein

Apoptotic cell death is a fundamental and essential process in development and tissue homeostasis of multicellular organisms. Roughly half of all neurons produced during neurogenesis die apoptotically before the nervous system matures. Apoptosis is a highly regulated biological process in which a cell is instructed to participate actively in its own demise. The signals identifying cells to be eliminated as well as the intracellular signaling events controlling apoptosis in the developing nervous system are far from being understood. This symposium will provide an overview on the current knowledge of cell-extrinsic and -intrinsic regulators of ontogenetic neural cell death.

Evidence for active triggering of neuronal death continues to accumulate. Death receptors such as p75, or FasR are thought to trigger cell death. Recent work by C. Henderson has provided new insights into the requirements for Fas signaling followed by a specific downstream pathway during motoneuron cell death.

The role of Fas ligand (FasL) is addressed by A. Martin-Villalba. Many neurological diseases involve neuronal degeneration and, consequently, cell death. Acute disorders, occurring within minutes and hours, e.g. brain trauma, or infarction involve injury-induced apoptosis. A. Martin-Villalba could demonstrate the neutralization of FasL is an essential step of her newly established experimental strategy to prevent ischemic neuron death in animal models of stroke.

Bcl-2 family members are important intracellular sensors that receive multiple signals from pathways upstream of irreversible cell damage. Bcl-family members play a pivotal role in deciding whether cells will live or die by either blocking or permitting the regulation of downstream cell death effectors at the mitochondrial level. J.-C. Martinou will address the molecular mechanisms underlying the mitochondrial involvement in apoptosis.

Cell death in the developing nervous system is already seen prior to neuronal differentiation and synaptogenesis. Early neural cell death is detected as early as neurulation and seems to affect proliferating neural precursor cells as well as young postmitotic cells during and following neurogenesis. Recent work by E. de la Rosa characterizes the molecular context in which cell death is permitted or prevented.

One of the still open questions relates to the cell extrinsic mechanisms that regulate cell death. Recent evidence provided by K. Krieglstein shows that the pleiotrophic molecule transforming growth factor-ß (TGF-ß) acts as a key regulator in the induction of developmental as well as lesion induced cell death.

79 Motoneuron Death through the Fas/NO pathway

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Death pathways restricted to specific neuronal classes could potentially allow for precise control of developmental neuronal death, and also underlie the selectivity of neuronal loss in neurodegenerative disease. We have recently identified a motor neuron-restricted cell death pathway that is triggered by the cell surface receptor Fas and which involves activation of Daxx, ASK1 and p38, transcriptional up-regulation of neuronal NOS together with the classical FADD/caspase-8 cascade. Motor neurons from transgenic mice for the ALS-linked SOD1 mutants G37R, G85R or G93A displayed increased cell death susceptibility to Fas-activation and NO but not to other death inducers such as trophic deprivation or glutamate receptor stimulation (Raoul et al. Neuron 2002). More recently we found that mutant SOD1 motor neuron death was amplified by a feedback loop through which NO up-regulates the endogenous Fas ligand (FasL) and led to increased S-nitrosylation of cellular proteins. No evidence for involvement of this pathway was found in other cell types such as cortical neurons, DRG neurons, cerebellar neurons or glial cells. In summary, these findings indicate that neuronal class-specific cell death programs can be sensitised by disease-related genetic factors and suggest that their signaling intermediates might represent new targets for drug screening in neurodegenerative diseases.

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80 The role of CD95-Ligand in the nervous system

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The CD95-Ligand (CD95L; Apo-1/Fas-ligand) is known to trigger apoptosis. Accordingly, in the adult CNS, CD95L expression increases under pathological situations such as stroke or spinal cord injury and induces apoptosis. Following stroke, infarct volumes in mice deficient in functional CD95L (gld) were significantly smaller than in wild-type (wt) animals. Also spinal cord injured animals treated with neutralising antibodies against CD95L, were capable of initiating active movements several weeks postinjury. In the embryonic brain CD95L is constitutively expressed in regions with detectable levels of apoptosis. However, absence of functional CD95L or CD95, as in gld and lpr mice respectively, did not affect the number of neurons in these regions. Interestingly, lpr mice exhibit atrophy of pyramidal neuron dendrites. In this line, we observed that CD95L induced neurite branching of young (1-4 days old) embryonic hippocampal and cortical neurons. CD95L triggered apoptosis only in older neurons (>6 days old). Thus, a signal downstream of CD95 can either induce apoptosis, as it is the case with the adult brain, or the remodelling of neurites as in the embryonic brain.

Apoptosis: Confusing the mitochondria. 81

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I will focus μ talk on two crucial events that occur during apoptosis at the level of mitochondria: 1) The permeabilization of the outer mitochondrial membrane; 2) The fragmentation of the mitochondrial network.

In response to various apoptotic stimuli, Bax, a pro-apoptotic member of the Bcl-2 family, oligomerizes and permeabilizes membranes to apoptogenic factors including cytochrome c. Bax oligomerization can also be induced by incubating isolated mitochondria containing endogenous Bax with recombinant tBid, *in vitro*. The mechanism whereby Bax oligomerizes under these conditions is still unknown. To address this question, recombinant human full-length Bax was purified as a monomeric protein. Bax failed to oligomerize spontaneously in isolated mitochondria or in liposomes composed of either cardiolipin or lipids extracted from mitochondria. However, in the presence of tBid, the protein formed large complexes in mitochondrial membranes and induced the release of cytochrome c. tBid also induced Bax oligomerization in isolated mitochondrial outer membranes, but not in other membranes such as plasma membranes or microsomes. Moreover, tBid-induced Bax oligomerization was inhibited when mitochondria were pretreated with protease K. The presence of the voltage-dependent anion channel was not required either for Bax oligomerization, or for Bax-induced cytochrome c release. Finally, Bax oligomerization was reconstituted in proteoliposomes made from mitochondrial membrane proteins. These findings implicate that tBid is necessary but not sufficient for Bax oligomerization: a mitochondrial protein is also required.

In addition to the permeabilization of the outer mitochondrial membrane, during apoptosis, the mitochondria fragment as a result of an imbalance between mitochondrial fission and fusion. We have identified the mammalian homologue of the yeast Fis1p known to participate in yeast mitochondrial division. This protein which we called hFis1, when over-expressed in various cell types, targeted to the outer mitochondrial membrane and induced mitochondrial fission. This event was inhibited by a dominant negative mutant of Drp1 (Drp1K38A), a major component of the fission apparatus. Fragmentation of the mitochondrial network by hFis1 was followed by the release of cytochrome c and ultimately apoptosis. Bcl-xL was able to block cytochrome c release and apoptosis but failed to prevent mitochondrial fragmentation. In contrast, Drp1K38A was able to prevent Fis-induced mitochondrial fragmentation but had no significant effect on survival. These data suggest that mitochondrial fragmentation is a dispensable event for at least certain apoptotic responses.

82 Regulation of programmed cell death during early neural development

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While the role of programmed cell death is established for connecting neurons, apoptosis during early neural development is little characterized. Neither a defined function nor the regulatory mechanisms of cell death affecting proliferating neuroepithelial cells and young neuroblasts are well known. In order to approach these questions, we first determined that TUNEL-positive cells are present in the mouse and chick embryonic retinas. Caspases, particularly caspase-3, are executors of retinal cell death, as shown by immunostaining for activated caspase-3 and by treatment with caspase inhibitors. Interestingly, *in vivo* inhibition of caspases in the chick embryo resulted in an increase in the number of retinal ganglion cells. Conversely, our previous observations had shown that inhibition of insulin signalling reduced the number of retinal ganglion cells. Both data together strongly suggest that cell death modulates the number of differentiating ganglion cells. The direct quantification of cell death on newly generated ganglion cells showed that this population is halved shortly after leaving the cell cycle. A further characterization of the insulin survival signalling has shown that in the embryonic mouse retina, as in the chick, all members of the insulin-related growth factor family are similarly potent preventing cell death induced by growth factor deprivation. The inhibition of phosphatidylinositol 3-kinase blocked this survival effect in the mouse retina. Altogether, our results confirm the relevance and precise regulation of programmed cell death during early neurogenesis and enforce the necessity of further define the role of this process in early neural development.

Related references:
Díaz et al. (2000). Development 127, 1641-1649. de la Rosa and de Pablo (2000). Trends Neurosci. 23, 454-458. Rubio et al. (2002). Eur J. Neurosci 15, 1646-1654.

83 TGF-β is a key regulator in ontogenetic neuron death

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Regulating the balance of neuron survival and death is a permanent issue in nervous system development, maintenance, degeneration and repair. Most recently, the pleiotrophic molecule transforming growth factor-β (TGF-β) has been shown to act as a key regulator in the induction of developmental as well as lesion induced cell death. TGF-ß induced apoptosis affects neurons of the peripheral and central nervous system, Schwann cells *in vivo* and oligodendrocytes *in vitro*. The role of TGF-β to regulate developmental cell death is now firmly established with evidence coming from *in vivo* models (chick and mouse), experimental (immunoneutralization) as well as genetic approaches (mouse knockout analysis). Furthermore, the role of TGF-β to regulate ontogenetic cell death is not restricted to nervous system development. Another well characterized apoptotic scenario is the morphogenetic cell death for tissue modeling and

for digit separation during limb formation. This talk will present the current knowledge on TGF-β induced cell death during nervous system development, as well as its underlying molecular mechanism.

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TGF-β modulated programmed cell death in the developing retina

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Programmed cell death (PCD) is a common mechanism of regulation of cell-number homeostasis in the organism. Transforming growth factors β (TGF-β) are expressed during the period of PCD of numerous neuron populations including the developing retina. We confirmed the pro-apoptotic role of endogenous TGF-β in the developing chick retina. Application of a TGF-β-neutralizing antibody to chick embryos in ovo resulted in a decrease of TUNEL-positive cells in the developing chick retina. We used *Tgfbeta 2-/-Tgfbeta 3-/-* double mouse mutants as a genetic model to consolidate the role of TGF-β2 and TGF-β3 in mediating PCD. Eye morphology is substantially changed in *Tgfbeta 2-/-Tgfbeta 3-/-* double null mouse mutants. The inner neural retina, the region where TGF-β receptor (TbetaR) I and II immunolocalization was most prominent, is considerably thickened in these animals. In line with these results, the number of TUNEL-positive cells was significantly reduced. To analyse the underlying intracellular signaling processes, we established a cell culture system mimicking the situation of TGF-β mediated ontogenetic cell death. Neutralization of TGF-β inhibits cell death of cultured chick retinal cells whereas exogenous application of TGF-β is followed by enhanced apoptosis. TGF-β induces the activation of c-jun N-terminal kinase in the MAP kinase pathway and provokes downregulation of the anti-apoptotic BCL-XL protein. Thus, TGF-β influences cell death via activation of a pro-apoptotic MAP-kinase cascade accompanied by a downregulation of anti-apoptotic signals. Our results consolidate previous findings that endogenous TGF-β is required to mediate PCD in developing retinal neurons *in vitro* and *in vivo*.

Apoptosis determines the ontogenetic regression of the cave fish eye

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The morphogenesis of the eye of the blind cave fish was examined using light and electronmicroscopical techniques. The troglobite cyprinid Phreatichthys andruzzii from Somalia is characterized by a strong degree of eye regression. The hatchling of this cave

fish exhibits a quite normal eye primordium with lens and nerve similar to teleost fishes with visual ability. The subsequent eye development is characterized by an early and rapid degenerative process beginning within the first day. The underlying mechanism of ontogenetic eye regression is programmed cell death, so called apoptosis. Excessive cell death occurs in Phreatichthys during a short apoptotic period, with the main organizational breakdown on the first day of development. Although retinal cytodifferentiation continues in the remaining cells, apoptosis predominates. Although precursors of retinal layers can be found, outer segments do not develop in the visual cells. Four weeks after hatching the eyes simply consist of strongly degenerated cysts. After two month any remnants of eyes are totally absent. The complete abolition of eyes is in contrast to other investigated cave fishes in which eye remnants in the adults are a regular feature. The extreme eye reduction has a strong impact on vision-processing brain centres. In adult Phreatichthys the optic tectum is reduced in size and the typical organization of tectal layers is absent.

86 The oxidative DNA damage and repair (p53) in rat brain aging.

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In the aging organism levels of free radicals increase, which leads to DNA damage, with 8-oxo-2'-deoxyguanosine (8-oxo2dG) being the most frequent mutagenic alteration. The cells respond to DNA injury by increased expression of p53 gene and p53 protein. Levels of 8-oxo2dG (HPLC/EC/UV), p53 mRNA (PCR) and p53 protein (Western blot) were estimated in four structures of rat brain, including grey matter (GM) of cerebral cortex, white matter of the brain (WM), cerebellum (C) and medulla oblongata (MO) of control (3.0 to 3.5 month-old) rats, 12 month-old rats and 24 month-old rats. The level of oxidative DNA damage which increased with age of experimental rats was noted in C (Mann-Whitney's test, $p<0.05$ in 12 month-old and $p<0.01$ in 24 month-old rats) and MO. In 12 month old animals the level of oxidative DNA damage in GM and WM was higher than in control animals. In parallel in control and 12 month-old animals p53 gene expression decreased while amounts of p53 protein increased, depending on the oxidative DNA damage. In 24 month-old rats, expression of p53 increased in all structures (Mann-Whitney' test: $p<0.05$ in GM, C, MO) while p53 protein showed decreased levels in most of structures of central nervous system (WM, C, MO).

Aging process leads to increased oxidative damage to DNA and augmented p53 gene expression, accompanied by a lowered expression of p53 protein.

Introductory remarks to Symposium 10

Insect neural and motor systems: from development to function and mechanics

Carsten Duch and Hans-Joachim Pflüger

The understanding of motor behavior and underlying circuitry requires a blend of many different research areas, making an integrative systems approach increasingly difficult. One of the strongholds of insect motor systems is that they can be analyzed at many different levels, and that bridging these levels is achieved in an increasing number of preparations. This symposium is intended to combine novel insights on the mechanisms underlying motor circuit development, the control of adult motor output by higher brain centers, the integration of motor output with muscle metabolism, and finally, the bionics underlying coordinated motor behavior.

Appropriate motor behavior relies on the integration of sensory information with the activity of central circuitry. However, isolated central networks can generate fictive locomotor rhythms in the absence of movement and sensory feedback. Therefore, the basic pattern of motor output is laid out by the intrinsic electrical properties and connectivity of neurons. Sensory input is required to adjust patterned motor output to changing environmental requirements. A central issue for our understanding of how locomotor circuits are specified and assembled is the extent to which sensory inputs are required as such systems develop. In his talk, Michael Bate (Cambridge, UK) will describe the effects of genetically eliminating sensory signaling or sensory structures on the embryonic and early postembryonic development of the peristaltic motor pattern of Drosophila.

Another aspect of genes being responsible for the formation of motor networks becomes apparent when postembryonic modifications of motor circuits follow a stereotypical developmental program with hormones acting as a timer. Hormonal control of postembryonic motor circuit remodeling is particularly apparent in holometabolous insects, such as Manduca and Drosophila. Among the genes that are directly activated by ecdysteroids is the Broad Complex (BRC). Christos Consoulas (Athens) will present recent data on the effects of BRC mutations on dendritic growth of an individually identified flight motoneuron during Drosophila metamorphosis.

Although ecdysteroids are the major player controlling motor circuit remodeling during insect metamorphosis, additional signals have important roles, too. A possible functional interplay between hormonal signals and activity-dependent mechanisms for structural and physiological changes of motoneurons will be addressed by Carsten Duch (Berlin). He will present data on the effects of selective electrical stimulations of identified motoneurons during Manduca development.

A particular feature of locomotory networks is their distribution over large parts of the central nervous system. ‘Higher locomotory centers’ in the brain may be important for the selection of motor patterns (‘motivation’), whereas segmental networks are important for controlling the rhythmical movements of limbs and joints with descending control necessary for a precise exertion of locomotory tasks. Roland Strauss (Würzburg) will show how mutations of different brain areas will differentially affect specific aspects of motor control inferring a modular control.

87 Neural networks and behaviour in the *Drosophila* embryo

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The *Drosophila* larva moves by peristaltic crawling. The central pattern generator for these movements is assembled in the embryo and begins to function several hours before the larva hatches. We are interested in the general question of how motor circuitry develops and we are using this simple system to ask how the necessary neural components are specified and assembled. We find that motor circuitry develops in the absence of sensory input, that the dendrites of the motorneurons are organised to form a myotopic map of the muscle field in each segment of the central nervous system and that synaptic input to these dendrites is cholinergic in origin. We are pursuing the question of how such dendrites are patterned and how synaptic specialisations are positioned and seeking to identify further essential components of the embryonic motor circuitry.

88 A steroid-regulated gene is required for dendritic growth of motoneurons during metamorphosis of *Drosophila melanogaster*

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The proper integration and transmission of information in the nervous system is based upon the formation of precise dendritic and axonal arborization patterns and synaptic connections during development. Modifications in dendritic architecture of post-synaptic partners, such as motoneurons, can either refine or, more likely, impair the function of motor circuits. In insects, many larval motoneurons survive to adulthood but undergo dendritic regression and outgrowth as they are incorporated into developing circuits. This remarkable example of dendritic plasticity has been explored at the level of individually identified motoneurons in *Drosophila*.

The motoneurons innervating the dorsolongitudinal indirect flight muscle of the adult are persistent larval neurons exhibiting two distinct metamorphic histories. The four of them are born in the embryo, innervate larval muscles and undergo dendritic regression and regrowth during metamorphosis. The fifth motoneuron (MN5) is also born embryonically, but remains developmentally arrested until the onset of metamorphosis. In the larva, MN5 lacks dendrites and its axon stops in the mesothoracic nerve without innervating a target muscle. During pupal development, MN5 undergoes *de novo* dendritic growth and extension of its axon to innervate the developing target muscle. The onset of dendritic growth occurs simultaneously for all five motoneurons at 20 hours after pupal formation (APF), the adult-like pattern of dendritic arborizations is established by 75h APF, but the growth of high order branches continues until adult emergence.

Metamorphosis is controlled by the steroid hormone, 20-hydroxyecdysone (20E). Among the genes that are directly activated by 20E is the *Broad Complex* (*BRC*). *BRC*

encodes zinc-finger-containing transcription factors (isoforms BRC-Z1 to -Z4) that are essential mediators of CNS reorganization during metamorphosis. In *BRC* mutants of the *2Bc* complementation group dendritic retraction occurs normally. However, for those *2Bc* mutants that survive to late pupal stages, the extent of dendritic arbors of the motoneurons and the cell body size of MN5 are small compared with stage-matched wild-type controls. Replacement of BRC-Z3 using a wild-type transgene rescues the dendritic and cell body defects of the motoneurons. Thus, BRC-Z3 isoform is required for proper dendritic growth of, at least, this particular group of motoneurons. The other three BRC isoforms are not required for either dendritic regression or outgrowth.

Titel Stage-specific activity patterns affect motoneuron structure during Manduca metamorphosis

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During insect metamorphosis, larval and adult behavior place clashingly different demands on the neuromuscular system. To accommodate changing behavioral requirements most larval muscles and sensory neurons are replaced by new adult elements, whereas most motoneurons persist metamorphosis and are remodeled to serve new adult functions. Remodeling of persisting motoneurons comprises larval synapse elimination, axonal and dendritic retraction during the last three days of larval life (W2-W4) followed by axonal and dendritic growth and adult synapse formation during pupal life (Duch et al., J Comp Neurol 422: 1-17, 2000). Since metamorphosis is governed by ecdysteroids, these neuromuscular changes have mostly been attributed to steroid control, although activity-depending mechanisms affect axonal sprouting in the *Drosophila* neuromuscular junction. To further address the role of activity during insect metamorphosis spiking patterns have to be determined and manipulated *in vivo*.

Therefore, chronic extracellular recordings were conducted *in vivo* from identified Manduca motoneurons throughout the last three days of larval life. During normal development of essentially intact animals, motoneuron activity was regulated stage-specific. Each of the late larval stages, (W2, W3, and W4) was characterized by stereotypic bursting patterns. A marked cessation in spiking activity during late W3 coincided with axonal regression, whereas ecdysis-spiking activity coincided with the onset of new terminal outgrowth.

Inducing ecdysis-like motoneuron spiking patterns at inappropriate times during normal development in intact animals resulted in dramatic outgrowth of their terminal arborizations, indicating that the normal decline of activity permitted retraction, whereas ecdysis patterns signaled new outgrowth. Metric analysis revealed that patterned stimulation induced significant increases in length, surface area, and volume of axon arbors (students T-test), whereas tonic stimulation did not affect any of these parameters. In addition to the marked effects of activity on axonal growth, preliminary results indicated that inappropriate spiking patterns affected also motoneuron dendritic structure, although such effects were limited to high order branches only. Therefore, activity-dependent

mechanisms act in concert with hormonal control of postembryonic motoneuron remodeling.

90 Control of *Drosophila* Walking and Orientation Behavior by Functional Subunits Localized in Different Neuropils of the Central Brain

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We have identified functional units in the control of walking and orientation behavior of *Drosophila melanogaster* and were able to locate several of these units in the brain. Special interest was taken in the role of the central complex - consisting of the four neuropils ellipsoid body (EB), fan-shaped body (FB), noduli (NO), and protocerebral bridge (PB) - and the mushroom bodies (MBs). We quantitatively assessed and compared the behavior of normal and mutant flies which had been isolated earlier either by behavioral or neuroanatomical means. Also included in the study were flies in which Gal4-directed neurotoxin expression suppressed proper function of particular neuropils of the brain, and flies lacking the mushroom bodies due to chemical ablation. The identified control units turned out to be surprisingly independent of each other: lesions in a particular brain neuropil affected specific control aspects but left most other functions largely intact.

The walking system can be genetically dissected into subsystems. Specific defects were found in either of the control systems for (1) the spatial placement of legs, (2) the temporal pattern of swing phases, (3) the range of stepping frequencies, (4) the swing phase duration, (5) the swing speed of legs, (6) the initiation and maintenance of step length, and (7) the across-body symmetry of step length. The differential control of swing speed of legs involves the PB of the central complex. It keeps the walking fly on target and at the same time optimizes the walking speed. PB-defective flies approach landmarks more slowly and in a less straight fashion than normal flies. Occlusion of a small frontal eye region in wild-type flies with light-tight paint creates a highly similar phenocopy of the PB mutant *no-bridge*. A hypothesis for the function of the PB based on this latter finding and on anatomical data will be discussed.

Visual orientation behavior has been studied with regard to landmark choice, course control and retreat behavior in a virtual-reality set-up. The central complex mediates the ability of normal flies to retain the direction toward a landmark *which became invisible during approach.* We now show that specifically the EB and the FB are involved. Lesions in the PB, the NO, or the MBs do not interrupt this ability. Structural EB or FB mutants quickly loose their bearings as soon as their chosen target landmark disappears from sight. More specifically, Gal4-directed inactivation of a group of ring cells in the EB destroys the after-fixation ability. But the affected flies are nevertheless able to properly steer towards *visible* landmarks. The MBs turned out to be necessary for the adaptive retreat from an initially attractive landmark which turns out to be inaccessible during the approach. Characteristic erroneously continued approaches at a water-filled moat were found in MB-less flies but never in any of the other test. In turn, choice beha-

vior of the MB-less flies and their ability to approach visible or hidden landmarks remain entirely normal. (Supported by a BMBF grant, FKZ: 0311855).

Fuel selection in locust flight muscle by the activity of neuromodulatory neurons. 91

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Insect flight comprises one of the most intense biochemical challenges known in nature, with extreme energy demands on the flight muscles. High rates of carbohydrate metabolism are necessary for take off, but trehalose reserves last only for few minutes of flight, and thus, muscles must switch to lipid metabolism. Biogenic amines affect glycolytic rates in nearly all classes of muscles and animals, but it is unknown how they are precisely targeted in open circulatory systems.

This study shows for the locust flight system that flight muscle glycolysis is affected by the activity of dorsal unpaired median (DUM) central neurons via the peripheral release of the biogenic amine octopamine. Glycolytic rate was measured by the amount of the main glycolysis regulator fructose 2,6 bis-phosphate (F26BP) in flight muscles that were exposed to octopamine released from DUM neurons versus control flight muscles from the same animals that were not exposed to DUM neuron activity. The activity of DUM neurons significantly increased the amount of F26BP in flight muscles. The cAMP-dependent protein kinase A is necessary but not sufficient for this neuronal control of muscle metabolism, suggesting a parallel control via a calcium dependent pathway. The activity patterns of DUM neurons during behavior imply that they ensure carbohydrate metabolism for take off, but stop to support glycolysis during flight. Indeed, DUM neurons innervating flight muscles are inhibited as soon as the wings open, but are active at rest. This is in contrast to DUM neurons supplying leg muscles which are activated as soon as the animal uses these muscles. We therefore conclude, that in contrast to the use of blood born factors in vertebrates, insect muscle metabolism can be directly controlled by the activity of central modulatory neurons.

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The Control of Vorticity in Flying Drosophila 92

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Insects were the first animals to evolve active flight and remain unsurpassed in many aspects of aerodynamic performance and maneuverability. Flies, in particular are capable of extraordinary aerial behaviours, aided by an array of unique sensory specializations including neural superposition eyes and gyroscopic halteres. Using such elaborate sensory input, flies steer and manoeuvre by changing many aspect of wing kinematics including angle of attack, the amplitude and frequency of the wing stroke, and the

timing and speed of wing rotation. The complex motion of insect wings produce fluid-mechanical forces that vary distinctly in both time and space. However, in contrast to ground-reaction forces produced by running animals, the physics of force production in fluids is complex and may depend on subtle changes in motion of the flapping fin or wing. To gain insights into the mechanisms of insect flight, we investigated the fluid-mechanical basis, the efficiency and the neuromuscular control of aerodynamic force production in flapping insect wings by conducting experiments in both a virtual-reality flight arena for *Drosophila* and a dynamically-scaled robotic wing in which aerodynamic forces and wake structure were analysed using various stroke kinematics. The dynamically-scaled robotic wing has revealed that the enhanced performance of insect wings result from an interaction of four distinct yet interactive aerodynamic mechanisms: delayed stall, rotational circulation, wake capture and wing interaction. The nature of these unsteady aerodynamic mechanism is quite diverse suggesting that insects employ a large variety of different aerodynamic phenomenons to enhance their locomotor performance. The analysis of these mechanisms currently broadens our understanding on both the evolution of sensory and neuronal structures necessary for flight and the development of a flight muscle system that may produce high mechanical power at elevated biomechanical efficiency but also allows rapid changes in power output and wing motion during fast flight maneuvers. Moreover, the discovery of unsteady aerodynamic mechanisms currently drives the construction of freely flying miniature-sized micro-robotic air vehicles that are propelled by flapping wing motion and exhibit enhanced aerial performance.

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93 *Multi compartment model* of developmental changes in dendritic shape during postembryonic motoneuron development

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During metamorphosis of the moth *Manduca sexta* the individually identified motoneuron MN5 is remodeled from a slow larval crawling into a fast adult flight neuron. A fivefold lower input resistance, a higher firing threshold, and an increase of voltage activated potassium current result in a much lower excitability of the adult MN5 as compared to the larval MN5 [Duch, Levine; 2000], thus meeting the newly acquired behavioural requirements. This postembryonic change in membrane properties and function is accompanied by drastic changes in dendritic architecture. Regression of larval dendrites is followed by growth and sprouting of new adult dendrites during metamorphosis [Libersat, Duch; 2002]. In many neurons processing of synaptic input and excitability are strongly dependent upon dendritic shape as demonstrated by studies that used multi compartment models.

We use the well defined changes in membrane properties, dendritic shape and behavioural function of MN5 to further address the question how developmental changes in

dendritic shape affect dendritic processing and excitability to accommodate changing behavioural requirements during postembryonic life.

Our model results from 3-D reconstructions of MN5 from confocal image stacks. We extract a skeleton and the diameters of the MN5 by means of our reconstruction method [Schmitt et. al, 2003]. These metric data are imported into the simulation environment GENESIS by means of an automated parser.

Currently, the simulations focus on adjustments and validations of our electrophysiological model in comparison to intracellular recordings in the cell body of MN5. Input resistance, the shape of passively conducted action potentials, resting membrane potential, firing threshold and time constant have been determined by single electrode current clamp recordings *in situ*. Under assumption of unaltered passive membrane properties, the length constant (λ) can be calculated by means of the model. In parallel we aim to determine λ by dual electrode recordings. We present simulation results concerning the following questions: First: In how far does an improved geometric model optimize the model, depending on various integration methods? Second: In what range do deviations of electrophysiological parameters influence the simulation results? Third: How does dendritic branch order affect summation of synaptic input with respect to the assumed site of spike initiation? Fourth: How does dendritic branch addition during development affect the summation of synaptic input in branches of given orders? Our model provides the possibility of synaptic inputs to specific branch orders.

In the long term we aim to understand to what extend changes in dendritic shape as occurring during normal postembryonic development may contribute to alterations in neuronal function that meet changing behavioural requirements.

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Changes in CaM kinase II activity and localization correlate with distinct phases of motoneuron dendritic growth during *Manduca* metamorphosis

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Developmental changes in intracellular calcium concentrations are critical for structural development of neuronal processes. Calcium-dependent enzymes like Calcineurin and CaM kinase II can translate changed calcium levels into alterations of the cytoskeleton or growth-cone morphology. However, little is known about the effects of interactions between developmental changes in calcium membrane currents, normal spiking activity and calcium-dependent kinase activity on structural modifications of dendrites. We aim to address the role of CaM kinase II activity for dendritic shape by using precisely staged alterations in the dendritic morphology of the motoneuron MN5 during the metamorphosis of *Manduca sexta*. MN5 undergoes drastic dendritic remodeling while it is changed from a slow larval crawling motoneuron into a fast adult flight-motoneuron (Duch and Levine, 2000, J Neurosci 20:6950-61). An initial retraction of the larval dendrites is followed by two distinct growth phases of new adult dendrites. In the first

growth phase all dendrites show prominent growth-cones and sprouting occurs in dendrites of all orders, while in the second phase the growth-cones collapse and further sprouting is limited to high order dendrites. This change in the mode of dendritic growth correlates in time with changes in calcium and potassium membrane currents, which in turn strongly affect the dendritic calcium levels upon spiking activity (Duch and Levine, 2002, J Neurophysiol 87:1415-25).

To address the role of CaM kinase II for dendritic growth we investigated its localization (immunohistochemistry) and its activity (phosphorylation-assays) during metamorphosis. In phosphorylation-assays we measured CaM kinase II activity which occurs naturally in the respective developmental stages (intrinsic activity) and the total amount of potential activity by activating with calcium (maximum possible activity). Intrinsic activity of CaM kinase II in the mesothoracic ganglion increased continuously as motoneuron calcium membrane currents increased between the last larval stage (L5) and pupal stage 8 (P8), but remained rather constant thereafter (P8 to P18/adult). Maximum possible activity of CaM kinase II was present in the ganglion at pupal stages when dendritic shape was modified. In contrast at stages of dendritic stability (larvae and adult) maximum possible CaM kinase II activity was reduced by up to 50%. Immunohistochemistry revealed parallel changes in the dispersion of CaM kinase expression during metamorphosis. In L5, there was a strong signal in growing axons and in some cell bodies, which ceased during early pupal life. In contrast, during early pupal stages immunostaining was weak in the neuropile but increased continuously until P8, thus correlating with the switch between the two different phases of dendritic growth. The observed developmental changes in the activity and in the localization of CaM kinase II make it a promising candidate that might be involved in translating developmental changes of dendritic calcium concentrations into structural changes. We are currently testing pharmacologically whether altered CaM kinase II activity impairs motoneuron dendritic shape during *Manduca* metamorphosis.

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95 Metric analysis of growth-cones during dendritic remodeling of an identified flight motoneuron in *Manduca sexta*

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During the development of dendrites growth-cones play a key role in path finding and in synapse formation. Therefore, growth-cone structure is important for correct neural circuitry formation. Common approaches to test for effects of signaling molecules or electric activity on growth-cone morphology and filopodia structure are electron microscopy or *in vitro* systems. Yet both attempts have drawbacks: Culturing withdraws neurons from their normal context and impairs their native morphology. Moreover, in cultured insect neurons axonal and dendritic parts can not be distinguished due to the lack of specific markers. Electron microscopy is very time consuming if applied for metric analysis of fine structures spread over an elaborate dendritic tree. Therefore, little is

known about dendritic growth-cone morphology during dendritic growth and synapse formation *in situ*.

We aim to quantify morphological key factors of a large number of growth-cones in an identified neuron at different developmental stages *in situ* to establish a database for the evaluation of future manipulation experiments. As model system we use the individually identified flight motoneuron MN5 of the holometabolic insect, *Manduca sexta*, which undergoes dramatic changes in dendritic structure during metamorphosis. Severe dendritic regression during the dismantling of larval motor circuits is followed by two distinct phases of dendritic growth during the integration of MN5 into the newly formed flight motor network (Libersat and Duch, 2002): An initial growth-cone-dependent (pupal stage P3 to P5) phase of dendritic growth and branching and a subsequent growth-cone-independent phase with new branch formation restricted to higher order dendrites only. Changes in growth-cone morphology were analyzed by 3D-reconstructions of high resolution confocal images. Morphometric analysis of growth-cones was performed with an optimized reconstruction module (S. Schmidt, M. Scholz, TU Berlin).

During the initial phase of dendritic growth and branching at the pupal stages P3 and P4 growth-cones were found to be exclusively localized at the tips of the dendrites. In contrast, after dendritic branching has progressed, growth-cones were also formed at internodial segments. Between the pupal stages P3 and P5 both filopodia number ($p<0.01$) and filopodia length ($p<0.001$) was decreased significantly. Thereafter, growth-cones disappeared and further dendritic branching was limited to high order dendrites only. Moreover, metric parameters of growth-cones, like filopodia length, differ among different compartments of the dendritic tree of MN5.

This morphometrical analysis of spatial and stage-dependent changes in dendritic growth-cone structure will serve as a backbone for future manipulation experiments to elucidate the role of hormones, activity and calcium-dependent mechanisms for postembryonic dendritic growth.

This work was supported by SFB515 and GRK120

Postembryonic growth of a first order interneuron in a developing sensory-motor circuit - A morphometric analysis

96

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Growing neurons have to meet several requirements when a functional adult network is established during development. Morphological and physiological properties change and thus might have an important impact on integration within dendritic compartments. To investigate processes underlying synaptic arrangement as well as changing integrative properties a detailed knowledge about 3D architecture and morphometric parameters of the growing dendritic tree is a fundamental prerequisite.

Marked morphological changes of afferences and synaptic rearrangement occur during development of the adult flight circuit of *Locusta migratoria*, and thus make the A4I1-system a good model to study developmental plasticity. In adults about 150 wind sensitive hair receptors located on the prosternum make monosynaptic connections to the ventral cord interneuron A4I1. In contrast only about 10 receptor cells are found in a first instar. Furthermore, during development activity dependent competition between receptor cells lead to axonal regression in particular receptor cells. Here, we aim to quantify structural changes of the A4I1 during normal, undisturbed development by using high resolution confocal microscpy and subsequent 3D-reconstruction.

In different larval stages the A4I1 and one identified receptor cell were co-labelled and scanned with a confocal microscope. Data were processed using 3D-visualization software Amira, where a self-programmed reconstruction module was incorporated.

Preliminary analysis was concerned with transition from third to fourth instar, when the highest number of receptor cells is added. During this moult the morphology of the A4I1 changed profoundly and in the fourth instar it resembles the adult morphology with prominent main neurites of large diameter. Morphometric analysis of input branches show that the neurite length increased twofold, the number of branches by about 50%. Branching was not restricted to highest order dendrites only.

Another difference concerns the overlap between axons from the receptor cells and the A4I1 dendrites. In earlier larval stages certain receptors show putative synaptic contact with anterior and posterior branches of the A4I1, whereas in adults connections occur only with posterior branches. Sensory axons ingrowing at an even later stage predominantly overlap with anterior dendrites.

The detailed knowledge about morphometric changes during undisturbed development will allow us to quantify the effects of manipulations on the forming network precisely, and how structural changes influence signal integration in A4I1 by means of detailed compartimental modelling.

This work was supported by DFG, BMBF 0311559 and GRK 120 "Signal cascades in living systems"

97 Transient potassium currents in identified subtypes of octopaminergic dorsal unpaired median (DUM-) neurons isolated from locust thoracic ganglia

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By the release of octopamine, neuronal activity of efferent dorsal unpaired median (DUM-) neurons modulates neuromuscular transmission and effects muscle catabolism. The projection and activation patterns allow to distinguish between different subtypes of DUM-neurons. At least two subpopulations of the locust thoracic DUM-neurons are shown to be differentially recruited in parallel to specific motor programs. With leg movements only DUM-neurons innervating leg muscles (DUM-5, DUM-3,4,5) are

activated. In contrast, DUM-neurons that supply flight muscles (DUM-3, DUM-3,4) are silent or inhibited even during flight behavior. Such specific synaptic activation requires a sufficient excitability determined by the intrinsic electrical membrane properties.

Therefore, we investigated the composition of voltage dependent ion channels (Na^+, Ca^{2+}, K^+) and current densities in previously identified subtypes of DUM-neurons. By axonal tracing with vital flourescent dyes, somata could be selectively labeled and isolated for short term cell culture in Leibovitz medium (L-15). Whole-cell patch clamp recordings showed that both sodium and calcium ions contribute to the inward current that build up the overshooting action potentials in the somatic membrane. On the other hand, a large and slowly inactivating transient outward current (TOC) could be released with (subthreshold) membrane potentials more positive than -50mV. These TOCs are generally known to speed up the repolarisation, to regulate the firing frequency and to modulate spike initiation during depolarization. Especially the latter could be demonstrated in DUM neurons.

Dependent on its state of inactivation ($V_{0.5}$ = -55mV) this TOC was shown to diminish the excitability due to counteracting the depolarising inputs. This was particularly obvious after hyperpolarising prepulses when this current becomes deinactivated. About half of the TOC was sensitive to cadmium (calcium dependent, IK_{Ca}). The cadmium-insensitive component (A-type, IK_A) activates and inactivates at less negative potentials (-40mV) and could be blocked by 4-Aminopyridine. Only a small amount of non-inactivating outward current (delayed rectifier, IK_{DR}) remained.

Whereas activation and inactivation kinetics did not differ between the DUM-neuron subtypes, we found significant higher TOC densities in DUM-neurons associated with flight muscles (DUM-3, DUM-3,4). A larger amount of TOC in these neurons would support inhibitory synaptic inputs to keep the neuron silent or suppress rebound activity during short releases from inhibition.

With support by the DFG

From Voxels to Model: Automatic Reconstruction of Neurons from Confocal Images

98

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In order to shed light on the complicated relation between physiology (function) and morphology (form) of neurons by means of compartment models, a geometrical exact reconstruction of their morphology is needed. We present here a complete framework to automatically reconstruct neuron-like structures, consisting of several processing steps. A preliminary skeletonization of the neural structure provides the initialization for the model-based reconstruction. The latter yields directly morphometric measurements for further analysis like dendrograms and compartment models which can be imported by modeling software like "Genesis".

Starting from a simple threshold segmentation we calculate a preliminary skeleton by surface shrinking. This provides a sufficient initialization for the topologically correct reconstruction of the neuron (without gaps and circles), for which the individual branches are modeled by Generalized Cylinders with circular cross-section. The shape of the axes and the radii at every point are fitted to the data by minimizing an energy-functional which incorporates the data evidence as well as a smoothness regularization. An elaborated user interface provides convenient control over the reconstruction process.

Additionally we developed a segmentation algorithm based on Geodesic Active Contours which softens the constraints of the Generalized Cylinders concerning the tree-like topology and the circular cross-section. It yields also a distance map helping to evaluate the proximity of the neural structure to other structures, allowing e.g. the analysis of synaptic markers w.r.t. a specific neuron [J. F. Evers, Proc. 29. Göttingen Neurobiology Conf., 2003]. For both the model-based reconstruction and the segmentation we developed an automatic adaption of the parameters.

The developed tools speed up the creation time needed for detailed reconstructions. The skeleton and the seamless determination of its diameters provides the basis for fast creation of detailed compartment models [S. Schönknecht, Proc. 29. Göttingen Neurobiology Conf., 2003]. This is crucial for comparative studies of a large number of neuronal models.

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99 Genetically linked formations of sensory and accessory components in the auditory system of Drosophila

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Hearing is a particularly sensitive form of mechanosensation that relies on the intricate arrangement of mechanosensory cells and an acousto-mechanical interface. The concerted action of these sensory and accessory components is clearly crucial for audition, raising the question of whether these components develop in a concerted way. Here, we report that in *Drosophila melanogaster*, the very gene that initiates the formation of sensory components -i.e. the auditory sensilla- is also required for the formation of accessory components -i.e. the auditory joint.

In *Drosophila melanogaster*, hearing relies on an antennal joint working in association with a chordotonal sense organ (CHO), Johnston's organ. The joint provides a flexible connection, allowing the distal part of the antenna to vibrate in response to sound and, thus, to serve as the sound receiver (Göpfert & Robert 2001, Nature 411, 908; Göpfert & Robert 2002, J Exp Biol 205, 1199). The receiver's vibration, in turn, mechanically activates the CHO (Eberl et al. 2000, J Neurosci 20, 5981). atonal (ato) is the proneural gene that initiates the formation of the auditory CHO (Jarman et al. 1993, Cell 73,

1307). Laser Doppler vibrometric measurements of the receiver's mechanical response in hemi- and homozygous ato1 mutants show that, in addition to eliminating the auditory CHO, loss of ato function makes the antennal receiver insensitive to sound, impairing its auditory function. Anatomically, the cause for this mechanical effect is identified to reside in the deprivation of specific exoskeletal joint structures; hemi- and homozygous ato1 mutant flies lack those cuticular membranes that guarantee joint flexibility and, thus, receiver mobility. Hence, ato, the *Drosophila* homologue of mouse Math1, is required for the formation of both the auditory CHO and joint, providing a genetic link between the sensory and accessory components that together transform fly antennae into ears.

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Mechanical activity of Drosophila mechanosensory neurons 100

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Evidence is accumulating that the auditory mechanosensory neurons of insects are motile and actively assist audition in a way analogous to that of cochlear hair cells in vertebrates (Eberl 1999, Curr Opin Neurobiol 9, 389; Göpfert & Robert 2001, Proc R Soc Lond B 268, 333; Robert & Göpfert 2002, Curr Opin Neurobiol 15, 715). To test this proposition, we have investigated the *in situ* mechanical properties of auditory mechanosensory neurons in *Drosophila melanogaster*, combining non-invasive mechanical analysis with genetic manipulations. We show that these neurons are motile; in addition to transducing sound-induced vibrations of the fly's antennal sound receiver, the neurons generate motions that mechanically drive the receiver and tune it to relevant sound.

Laser vibrometric analysis of the receiver's mechanics revealed the presence of an active auditory tuning mechanism. In wild-type flies, the receiver's mechanical response is nonlinear; the receiver's resonant tuning shifts down in frequency as intensity declines, moving towards those low frequencies that dominate the flies' songs. As expected from an active tuning mechanism, this stiffness-dominated nonlinearity is physiologically vulnerable. Transient exposure to carbon dioxide reversibly linearizes the receiver's mechanical response. In the absence of sound, in turn, the receiver of wild-type flies twitches spontaneously, demonstrating that the receiver's nonlinearity associates with mechanical activity. A motion generating mechanism tunes fly ears to fly songs.

Genetic dissection of the receiver's mechanics shows that the motion generating mechanism resides in the fly's auditory mechanosensory neurons. Disconnecting the mechanosensory neurons from the antennal receiver (Chung et al. 2001, Neuron 29, 415), mutations in nompA eliminate the nonlinearity and spontaneous oscillation activity in the receiver's mechanics. This means that the receiver's mechanics itself is linear and passive; nonlinearity and oscillation activity are introduced by motility of mechanosensory neurons. This neural origin of auditory motion generation is supported by the partial deprivation of the receiver's nonlinearity and oscillation activity caused by mutations in

nompC. This gene encodes one of the mechanotransduction channels expressed in the auditory mechanosensory neurons (Walker et al. 2000, Science 287, 2229). Complete loss of the receiver's nonlinearity and oscillation activity, as observed in nompA mutants, also results from mutations in btv and tilB. Mutations in btv lead to structural aberrations of dendritic cilia, whereas tilB mutants, in addition to being death, fail to produce motile sperm (Eberl et al. 2000, J Neurosci 20, 5981). The complete loss of auditory motion generation in these mutants thus suggests that motion generation by *Drosophila* auditory mechanosensory neurons involves the mechanical activity of dendritic cilia and ciliary motors, pointing to an uncanny resemblance between the motility of insect auditory mechanosensory neurons and the motility of sperm.

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101 From the NMJ into the CNS - Synapse and circuit formation in fruitflies

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Insect neurons are individually identifiable and have been used successfully to study principles of the formation and function of neuronal circuits. In the fruitfly *Drosophila* the advantages of identifiable neurons can be combined with efficient genetic approaches. We want to capitalise on this potential and study *de novo* formation of synapses and neuronal circuits in the embryonic *Drosophila* CNS. As a prerequisite we need to a) describe principles of the structural organisation of the *Drosophila* CNS, b) identify genetically amenable neurons which can be used for our studies, and c) develop methods and strategies to unravel mechanisms of synapse or circuit formation.

We will present data showing that *Drosophila* neurons form different pre- and postsynaptic compartments (Löhr et al., 2002, J. Neurosci. 22, 10357ff.). Thus, we can define primary neurites (axons), neurites harbouring output synapses, and neurites which are exclusively postsynaptic (showing similarities to vertebrate dendrites). In order to investigate mechanisms underlying the fomation of these compartments we have carried out different kinds of projects: i) Genetic mosaic studies which are either based on transplantation of neuronal precursor cells or based on targeted mis-expression of genes in a few identified neurons (Gal4/Uas-system; Brand and Perrimon, 1993, Development 118, 401ff.). ii) In order to facilitate analyses of the three-dimensional structure of neuronal processes in the synaptic neuropile, we have adopted and standardised immunological marker expression which can be used as landmarks for the charting and classification of neurites. iii) By using this mapping procedure we have carried out precise descriptions of genetically amenable (Gal4-expressing) neurons, which can now be used for experimental approaches towards an understanding of synapse and/or circuit formation. iv) In addition to studies in the embryonic/larval CNS we can also raise neuronal cell lineages in embryonic primary cell cultures (Küppers et al., 2003, J. Neurochem., in

press), i.e. deprive them of their cellular contexts in order to address signal dependence *vs.* cell autonomy of synaptic differentiation processes. Our culture conditions are based on standard Schneider's medium but were modified in a way that these cell cultures acquire mature properties (synaptic differentiation, transmitter expression, neuronal activity) and, in addition, show quite normal lineage compositions so that some model neurons previously identified *in situ* can be recognised even in culture. v) Capitalising on these insights, techniques and tools, we have now begun to address mechanisms underlying synapse and circuit formation, and several examples will be presented.

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Towards the Neuronal Substrates Underlying Insect Climbing Behavior - A High-Speed 3D-Video Analysis of Normal and Mutant Fruit Flies 102

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We are investigating the climbing abilities of normal and mutant *Drosophila melanogaster* strains to analyze the underlying neuronal substrate. Flight-incapacitated flies are confronted with a gap of variable size in the walkway. Their behavior is monitored by two high-speed cameras from the side and from above. Flies attempt to cross gaps much wider than their own body length of 2.5 mm. A comparison of seeing and temporarily blinded wild-type flies revealed a strong visual motivation. Normal-sighted flies accomplish a much higher success rate than visually impaired flies (e.g. 70% versus 5% at gaps of 3.5 mm width). Neurogenetics provides the tools to study the translation of the visual information into climbing motivation via a yet unknown visuo-motor interface and ultimately into successful climbing behavior.

The motivation and ability to climb over gaps is solely conveyed by the principal receptor system R1-R6. A study of *sev* (R7 missing, R8-input greatly reduced) versus *ort[1] ninaE[1]* mutant flies (R1-R6 degenerated) revealed that the color vision system is not involved. The major photoreceptors R1-R6 are necessary and sufficient for proper gap crossing. Motion vision is likely involved when it comes to distance estimation.

Typically, wild-type flies cross as follows. (1) Both front legs step into the gap. (2) The fly then leans forward into the gap while all legs stay on the ground. (3) With its thorax and legs motionless, the fly slowly lifts and lowers its abdomen once. (4) Middle and hind legs are then repositioned. The fly leans further out and attaches its middle legs at the proximal vertical wall well below the edge. The middle legs extend, the head is pressed up. (5) In this posture the front legs perform far-reaching search movements until (6) they finally make contact with the distal rim. (7) Now, both middle legs swing over to the distal side surface. (8) Next, the hind legs are being released and swing over together with the abdomen. Middle and front legs pull the body towards the wall. (9) Without respite the fly then climbs up the distal side surface and around the edge, where it continues walking.

Using structural brain mutants and Gal4-directed expression of neurotoxins we are investigating the involvement of certain brain areas of the fly in climbing control such as the protocerebral bridge and the ellipsoid body of the central complex. All investigated protocerebral-bridge mutants *C141*, *cex[KS181]*, *ey[JD]*, *nob[KS49]*, and *oc[1]* perform poorly when it comes to wider gaps. The same applies to both of the ellipsoid-body mutants studied up to now (*ebo[678]*, *ceb[849]*). In contrast, the mushroom bodies of the fly brain, known for their function in olfactory learning, seem not to be involved. Structural mushroom-body mutants (*mbm[N337]*) and flies with partially blocked mushroom-body synapses (Gal4-mb247/TTX) show a normal performance. Various mutant strains, originally isolated for walking deficits on a smooth horizontal surface, are currently under investigation. Their performance ranges from entirely normal to complete incapacity. (Supported by BMBF, FKZ: 0311855)

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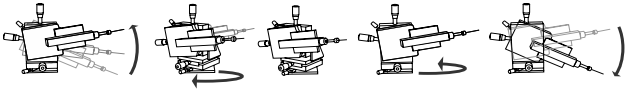

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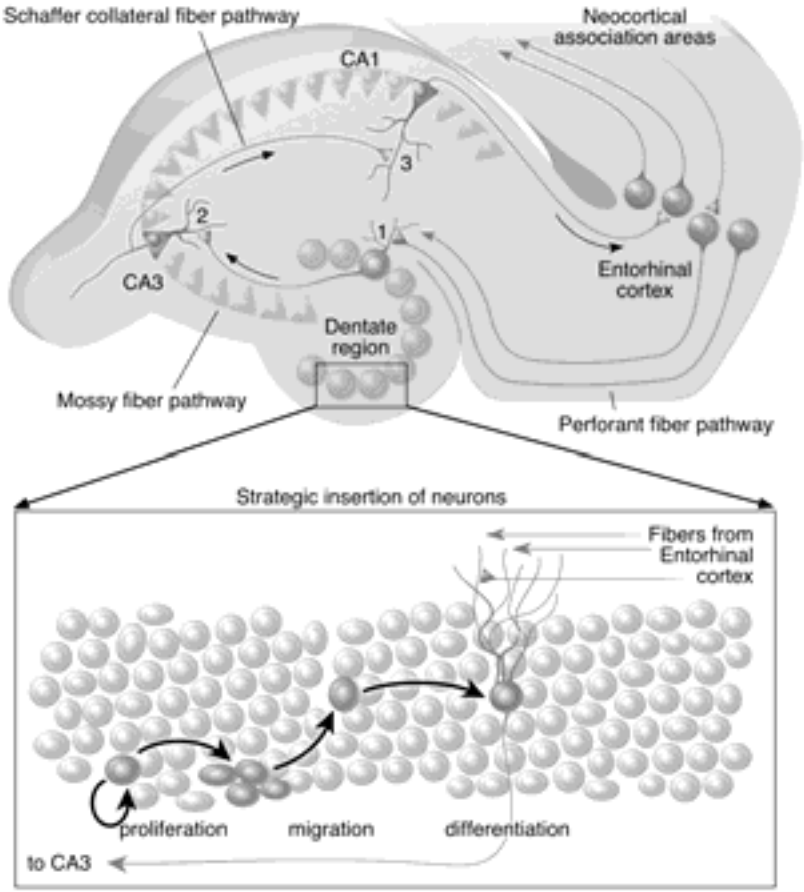
Schaffer collateral fiber pathway
Neocortical association areas
CA1
3
2
1
CA3
Entorhinal cortex
Dentate region
Mossy fiber pathway
Perforant fiber pathway
Strategic insertion of neurons
Fibers from Entorhinal cortex
proliferation
migration
differentiation
to CA3

Introductory Remarks to Symposium 11

Adult neurogenesis

Gerd Kempermann

The adult brain generates new neurons throughout life. However, it seems to do so only in two privileged regions in the olfactory system and in the hippocampus. In astonishing contrast to this, stem or progenitor cells can be found in the entire adult brain. Potentially they could give rise to new neurons, because they do so after propagation in vitro. In the adult hippocampus, neurogenesis underlies a complex, activity-dependent regulation. First theories attempt to place adult neurogenesis into functional contexts. How can new neurons and thus neural stem cells contribute to hippocampal function? Is adult neurogenesis necessary for the function of the adult hippocampus? And what about the apparently quiescent stem or progenitor cells outside the neurogenic regions? There is increasing evidence that under certain conditions reactive neurogenesis is possible from these cells. However, the adult brain does regenerate poorly and does not seem to make use of the potential it harbors. Why is that so? Will it be possible to promote regeneration from these cells? And would they be functionally relevant? Surprisingly, adult neurogenesis is linked to angiogenesis and bone marrow derived cells can form neurons in the adult brain. It is not clear whether bone marrow-derived brain cells reflect a fundamental biological principle or occur only under experimental conditions. In any case, adult neurogenesis and neural stem cell biology in general are much more complex than previously thought. The symposium is designed to address and discuss some of the topics of neural stem cell biology that have changed or will likely change fundamental neurobiological concepts.

The symposium begins with an overview on adult hippocampal neurogenesis, the role of neural progenitor cells in it and on how new neurons might contribute to hippocampal function. H. Georg Kuhn will introduce adult neurogenesis in the olfactory system. He will discuss, which role cell death plays in adult neurogenesis and will show new evidence, how intricately adult neurogenesis is linked to angiogenesis. Josef Priller will talk about the findings that at least under certain conditions bone-marrow derived cells can give rise to neurons and microglia in the adult brain. These data provoke very profound questions on the nature and origins of cellular plasticity in the adult brain. Among the human disorders which might be linked to stem or progenitor cell activity in the adult brain, temporal lobe epilepsy is of particular interest. Otmar Wiestler will present investigations on human hippocampal tissues, revealing that in the hippocampus of patients with temporal lobe epilepsy changes can be found that could be predicted from animal studies of adult hippocampal neurogenesis under the conditions of experimental seizures. This unique opportunity notwithstanding it remains difficult to study adult neurogenesis and neural stem cells in general in the adult human brain. In his presentation Mathias Höhn will explain new ideas on how new imaging technologies will allow us to visualize cellular plasticity in the living adult brain.

103 From progenitor cells to new neurons in the adult brain: Possible functions for adult hippocampal neurogenesis

Gerd Kempermann

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The adult mammalian hippocampus is special in that it makes uses of an otherwise neglected mechanism of cellular plasticity. New neurons are generated in the adult dentate gyrus, and this process is regulated in a suggestive activity- and experience-dependent manner. Only few details are known about the mechanisms underlying this regulation, but it has become clear that the process is controlled on several steps of neuronal differentiation and is governed by different inherited traits.

By the standard of proliferative activity and the expression of intermediate filament nestin two populations of progenitor cells can be found in the adult murine hippocampus. Surprisingly, it is only one of these populations that response to the stimuli that are mediating the neurogenic effects of for example physical activity and environmental enrichment. Several steps of neuronal development originating from these cells can now be distinguished *in vivo*. Within that development we have found that the decision for a new neuron to develop is made early. Also, new neurons are very stable and remain in their acquired location in the dentate gyrus for a long time. At the same time, a much larger group of cells show signs of immature neurons, possibly reflecting the pool, from which more new neurons could be recruited, should hippocampal function require this.

We have found that the genetically determined baseline level of adult hippocampal neurogenesis correlates with parameters describing the acquisition of a hippocampal learning task. Based on this and similar observation we have developed the hypothesis that adult hippocampal neurogenesis serves in optimizing the strength of the mossy fiber connection between the dentate gyrus and region CA1.

Taken together we have come closer to identifying the key points in adult hippocampal neurogenesis, at which the regulation determined by underlying genes and environmental and activity-dependent stimuli act upon each other in order to functionally recruit a new neuron into the hippocampal network.

104 Adult neurogenesis: A balance of proliferation and cell death

H. Georg Kuhn

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Neural stem or progenitor cells can be isolated from several regions of the adult mammalian brain, but only two distinct regions of the mature brain continue to produce new neurons *in vivo*. Proliferating neural stem cells and their progeny in the olfactory bulb and dentate gyrus allow us to study the development of nerve cells from proliferation and migration to full differentiation and functional integration under *in situ* conditions in the adult brain. As yet, it remains unresolved if and how these new neurons contribute to brain functions; however, several possible regulators of adult neurogenesis have been

described. More general stimuli, such as environmental stimulation and physical activity, as well as pathological conditions like stroke, brain lesions and seizures can have a profound stimulating effect on the generation of new neurons. On the molecular level, neurotransmitters, growth factors and transcription factors appear to be effective in regulating stem cell activity *in vivo* and *in vitro*. The continuous production of neurons in the adult brain also raises the hypothesis of whether an apoptotic elimination mechanism may counterbalance neurogenesis and regulate the size of neuronal populations. The high number of apoptotic profiles, which can be found in regions of neurogenesis, support the view that neurogenesis could be part of a continuous neuronal replacement mechanism in the adult brain.

To make use of the endogenous stem cell population for therapeutic strategies, we need to understand the mechanisms that underlie the continuing neurogenesis in the adult brain. Our strategy is to manipulate stem cell proliferation and/or cell death *in vivo* in order to analyze whether this has an effect on the number of newly generated neurons. We use growth factors and transgenic/knock-out technology to determine whether neurogenesis in the adult brain is altered in response to these molecular changes. Individual aspects of these studies include proliferative changes and altered differentiation of neural stem cells after growth factor infusion or in animals with null-mutations for genes controlling cell cycle and neural fate determination.

This work is supported by the Volkswagen-Stiftung.

Engraftment of bone marrow-derived cells in the murine CNS 105

Josef Priller

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The central nervous system (CNS) has low regenerative potential. Interestingly, recent experimental evidence in bone marrow (BM) chimeras suggests that blood borne cells are able to enter the CNS and to differentiate into glial cells and neurons. In particular, the use of the green fluorescent protein (GFP) as a marker of BM cells has helped to track donor-derived cells in the brain after bone marrow transplantation. Thus, microglia were found to engraft throughout the adult CNS, and some researchers have also detected astrocytes derived from the donor BM. Perhaps the most astonishing finding was the presence of GFP-expressing neuronal phenotypes and Purkinje cells in the brains of mice transplanted with GFP-marked BM cells. The data suggest that the bone marrow compartment may provide a source of cells capable of repair in the damaged CNS.

Evidence for neurogenesis in human temporal lobe epilepsy 106

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Abstract has not been submitted.

107 How to track neurogenesis and stem cell activity in the adult brain

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In vivo monitoring of stem cells after grafting is essential for a better understanding of their migrational dynamics and differentiation processes and of their regeneration potential.With new developments in labeling stem cells *in vitro* for MRi visualization and immense sensitivity increases in MR microscopy, stem cells, implanted into rodent brain can be followed with MR imaging *in vivo*. Thus the dynamics of these cells in relation to cerebral lesions are observed over time. Under conditions of focal cerebral ischemia, embryonic stem cells migrate towards the lesion periphery where a massive accumulation of cells is registered within days. These cells differentiate into neurons and glia, with only very few oligodendrocytes. Synaptophysin immunohistochemical staining shows synapto-neogenesis of the implanted, fully differentiated ES cells, thus indicating beginning formation of (functional) networks in the periphery of the ischemic lesion. It will be shown that the methodological approach is ideally suited for the noninvasive observation of cell migration, engraftment, and morphological differentiation at high spatial and temporal resolution.

108 The ecto-ATPase NTPDase2 is expressed in the germinal zones of the developing and adult rat brain

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During prenatal and early postnatal stages neurogenesis takes place in two actively proliferating zones, the ventricular zone (VZ) and the subventricular zone (SVZ), two relatively thin layers lining the primitive ventricular cavities. After birth these layers progressively disappear. However, in the anterior part of the lateral ventricle, subependymal layers of tightly packed cells persist with the potential to proliferate and to form neurons and glia. The adult SVZ and its rostral extension generate neuronal precursors that migrate towards the olfactory bulb (OB), where they differentiate into neurons. The migrating neuroblasts are surrounded by a sheath of astrocytes (type-B cells). Using immunostaining, *in situ* hybridization and enzyme histochemistry, we demonstrate that the ecto-ATPase nucleoside triphosphate diphosphohydrolase 2 (NTPDase2) is ex-

pressed in the subventricular zone and the rostral migratory stream of the adult rat brain. NTPDase2 is a member of the E-NTPDase family containing a transmembrane-domain at the N- and C-terminus, respectively and a large extracellular loop with the catalytic site. This enzymes hydrolyze extracellular nucleoside triphosphates to the respective nucleoside diphosphates and are thought to directly modulate ATP receptor-mediated cell communication. Double labeling for the astrocyte intermediate filament protein GFAP and the glial glutamate transporter GLAST identifies the NTPDase2-positive cells as type-B cells. During development the enzyme protein is first detected at E18, long before expression of the astrocyte marker GFAP. It gradually becomes expressed along the ventricular and subventricular zone of the brain, followed by complete retraction to the adult expression pattern at P21. NTPDase2 is transiently expressed in the outer molecular layer of the dentate gyrus and within the cerebellar white matter and is associated with select microvessels, tanycytes of the third ventricle, and subpial astrocytes of the adult brain. Our results suggest that NTPDase2 can serve as a novel marker for specifying subsets of cells during *in vivo* and *in vitro* studies of neural development and raise the possibility that ATP-mediated signaling pathways play a role in neural development and differentiation.

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Introductory Remarks to Symposium 12

Invasive recording from the human brain – linking clinical applications with neurobiological research

Andreas K. Engel and Christian E. Elger

Currently, the vast majority of physiological data about the human brain are obtained by means of non-invasive methods, particularly functional MRI, EEG and MEG. These methods do not provide a sufficient resolution to permit the observation of physiological processes at the level of single cells or small cell assemblies. Therefore, our knowledge about physiological processes at the cellular level is largely inferential and based on comparative data from animal models. However, as part of therapeutical approaches it is in some cases possible, based on well defined clinical indications, to obtain data from invasive recordings in the human brain. This holds, e.g., for patients with neurodegenerative diseases of the basal ganglia (such as Parkinson's disease) or with epilepsies that are resistant to pharmacological treatment. In such cases, invasive recordings can be an indispensable means for both diagnosis of the respective disorder and for defining the appropriate therapeutical approach. Methodologically, this implies the use of electrodes for recording local field potientials reflecting the coherent activity of small cell assemblies, or even that of microelectrodes providing single-cell activity. In addition to their diagnostic relevance, such data are crucial for understanding the pathophysiology of the respective disorders and for linking animal models to the respective human disorders. Moreover, they can provide insights into basic mechanisms of normal brain functions such as movement control, sensory representation or memory formation. The goal of the symposium is to provide an overview of current developments in this field and to highlight approaches that link clinical applications with basic neurobiological research. Elger will introduce the approaches used in this research area and discuss the relevance of invasive methods for diagnosis of epilepsies. Subsequently, Engel et al. and Brown will focus on recordings in patients with movements disorders. The talk by Fernandez will review results on memory formation obtained in patients with epileptic disorders. Lachaux will present results on high-frequency oscillations during face perception in epileptic patients. Finally, Fried will discuss data that are relevant to understanding the role of single neurons in object representation in the human medial temporal lobe.

109 Microelectrode recordings from the human basal ganglia

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Intraoperative microelectrode recordings allow unique closeup views of the processes underlying pathological brain function at a neuronal level. A new approach to understanding basal ganglia function is inspired by observations suggesting that temporal patterning and coherence of neural signals may have a key role in the processing carried out by these structures. An increase in synchronization between neurons has been proposed to be an important pathophysiological principle in hypokinetic movement disorders. Detection of synchronized neuronal activity requires the analysis of the temporal relations among the discharges of simultaneously recorded neurones. To this end, tetrode recordings provide a valuable tool since tetrodes allow spike separation with much higher yield than conventional single lead microelectrodes. In addition, they allow reliable measurements of single-cell firing rates.

We have recorded single-unit activity extracellularly with a multifiber tetrode (Thomas Recording). Recordings were performed during stereotactic surgical treatment in 3 awake patients with advanced Parkinson´s Disease (PD) undergoing STN stimulation (mean age 63 y) as well as 3 anesthetised patients with dystonia (pallidal stimulation; mean age 35 y). Neuronal activity was amplified, bandpass filtered and digitized for offline-analysis. Separate spike-waveform clusters were identified manually, based on spike amplitude, total energy or projection onto 1st and 2nd principal component. For each isolated neuron firing rate histograms were constructed. Moreover, autocorrelation (ACF) and crosscorrelation functions (CCF) were computed to study discharge patterns and neuronal interactions.

In PD patients, 61 single units were isolated from 29 recording sites. The average yield of isolated units differed only slightly across structures: Thalamus (8|5, mean 1.6), STN (42|19, mean 2.2), SNr (11|5, mean 2.2). In Thalamus and STN, but not SNr, center peaks were observed in a substantial fraction of ACFs indicating bursting of the recorded neurons. In the STN, typical burst duration was in the range of 130ms. Cross-correlation analysis did not yield evidence for synchronous firing. In patients with dystonia, 129 isolated single-units were obtained from 52 recording sites. The incidence of isolated units was comparable for the recorded structures: Putamen (29|12, mean 2.4), GPe (14|40, mean 2.9), GPi (26|60, mean 2.3). The incidence of modulated ACFs was higher for GP neurons than for cells in the Putamen. In GPe and GPi, peak widths were in the range of 50-200ms and 40-120ms. Occasionally, an oscillatory modulation was observed. In most cases, cross-correlograms were flat.

The data clearly demonstrate that tetrode recordings yield superior quality in terms of single-unit separation and, thus, allow more reliable estimates of single-unit firing rates than conventional electrodes. Our data show, at least in part, lower firing rates than

previous studies. Conventional techniques do not allow completely reliable sorting and, hence, may overestimate actual firing rates. Analysis of the temporal patterning revealed a high variability in discharge patterns (bursting behaviour) of neurons across and within structures. Interestingly, cross-correlation analysis did not provide evidence for synchronized activity between neighbouring cells. Our data demonstrate the usefulness of the approach for the evaluation of pathophysiological hypotheses about changes in firing rates and neuronal interactions in basal ganglia disorders.

Task-related coherence in Parkinson's Disease. 110

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Recordings from macroelectrodes implanted as part of the surgical management of patients with Parkinsons disease afford a unique opportunity to discern the role of neuronal synchronisation in the function of the human basal ganglia. Using this technique subjects can be investigated while alert and engaged in a variety of motor tasks. The recorded local field potential (LFP) changes represent momentary variations in the degree of synchronisation between neuronal elements. Here we present evidence that changes in the degree of rhythmic synchronisation within the basal ganglia are linked to the preparation and execution of movement, and are critically dependent on the prevailing level of dopaminergic activity.

First, we simultaneously recorded LFPs from the subthalamic nucleus (STN), ipsilateral globus pallidus interna (GPi) and scalp EEG during voluntary movements of a hand-held joystick in awake patients following neurosurgery for Parkinsons disease. Without medication the power within STN and the coherence between STN and GPi was dominated by activity with a frequency <30 Hz. This coupling was attenuated with movement. In the presence of exogenous dopaminergic stimulation, power within STN and coherence between STN and GPi was dominated by activity at 70 - 85 Hz that increased with movement. The movement related changes in coherence between STN and EEG showed a similar pattern of pharmacological dependence as seen subcortically.

These studies only showed unequivocal premovement changes in oscillatory activity in a warning-go paradigm, in which variations may have related to alterations in general attention following the warning cue. We therefore recorded LFPs from the STN of parkinsonian patients during the performance of a precued reaction task in which the cue either predicted or failed to predict the demands of the imperative go signal. Modulation of oscillatory LFP activity prior to the movement and shortening of reaction times was greater following predictive rather than unreliable warning cues. Thus the spectral changes occurring prior to movement are at least partly related to movement preparation.

Overall, the findings indicate that frequency-specific changes in synchronisation occur in the basal ganglia with respect to movement preparation and execution. These changes are partly dependent on the level of prevailing dopaminergic activity and extend to involve subcortico-cortical motor loops, as befitting a process that may be mechanistically important in the organisation of voluntary movement.

111 Rhinal-hippocampal coupling during human memory formation

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The integrity of the medial temporal lobe (MTL) is essential for declarative memory, the ability to store and recall consciously events and facts. In the studies presented here, we explored neural activity during declarative memory formation by invasive EEG recordings from bilateral MTL depth electrodes in epilepsy patients. To obtain normal electrophysiological correlates of memory formation, only activity from the MTL not affected by epilepsy was included into our analyses. In the initial study, we compared event-related potentials (ERPs) elicited by items that were, or were not, successfully recalled in a subsequent free recall test. Two subsequent memory effects were observed in ERPs from either the rhinal cortex or the hippocampus. Larger activity was associated with subsequently recalled items in both MTL substructures. An onset latency difference of 200 ms and a positive correlation between the sizes of these two ERP effects suggests a serial processing hierarchy. Enhanced phase-synchronization of induced γ oscillations between rhinal and hippocampal activity for successful memory formation confirmed this interaction and identified a potential mechanism underlying this rhinal-hippocampal coupling. The enhanced γ phase synchronization is accompanied by an enhanced rhinal-hippocampal coherence in the θ band, suggesting a close interaction of both mechanisms during declarative memory formation. In a second ERP study, contrasting words with high and low frequency of usage, disappeared the correlation between the sizes of the rhinal and hippocampal subsequent memory effects. Replicating our initial findings, high frequency words elicited an early rhinal and a later hippocampal subsequent memory effect. Low frequency words with a smaller lexical-semantic context, elicited in contrast no rhinal but the hippocampal effect. Rhinal processing seems therefore to reflect a semantic operation supporting memory formation indirectly, while hippocampal processing seems to reflect a specific mnemonic operation of declarative memory formation. While rhinal-hippocampal γ synchronization may be closely related to the actual mnemonic process of declarative memory formation by enabling fast coupling and decoupling of both MTL substructures, θ coherence might be associated with a slowly modulated coupling associated with an encoding state.

112 Increase of High-Frequency (>150 Hz) Intracranial EEG Activity during Face Perception in Humans

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We have analyzed high-frequency components of the intracranial EEG activity in response to visual stimuli in the occipital and temporal cortex of epileptic patients. Face perception in particular elicited modulations in the γ band at frequency ranges up to 250

Hz. This study suggests that the response to complex visual stimuli extends well beyond the common frequency components usually studied in evoked potentials.

Dynamics of single neurons in the human medial temporal lobe during perception and memory tasks

113

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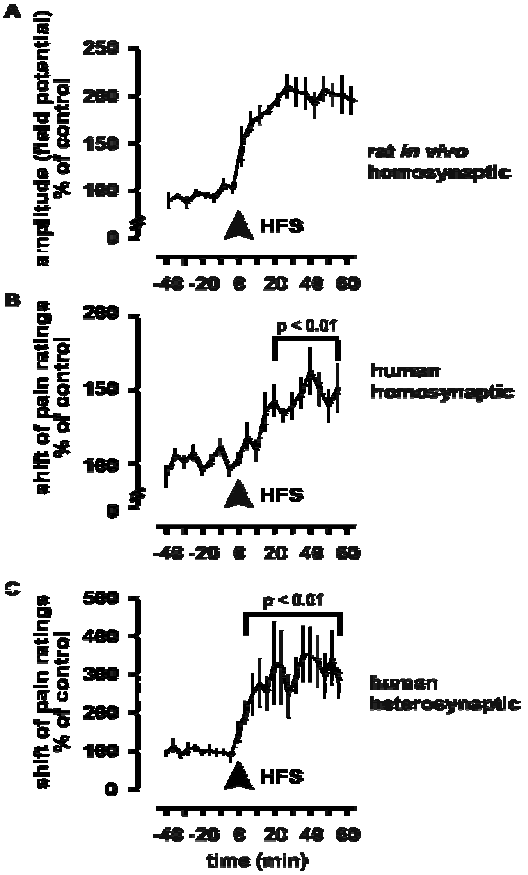
The human medial temporal lobe plays a major role in transforming present experience into future conscious recollection. We have recorded directly from single neurons in the hippocampus, amygdala and parahippocampal regions during perception and memory tasks. These tasks have included flash suppression, delayed nonmatch-to-sample memory tasks, paired associate learning, imagery, and spatial navigation. Recordings were performed in patients implanted with intracranial depth electrodes to identify the seizure focus for potential surgical resection.

These recordings show that the activity of single neurons reflect the percept rather than the image falling on the retina. Furthermore, this activity appears to be selective to stimulus category, such as faces or places, and to a specific individual stimulus, such as an individual person. The activity during encoding predicts future recollection. Furthermore, the activity during visual recall or imagery recapitulates neuronal activity during encoding.

The activity of single neurons in the human hippocampus and environs reflects, therefore, abstraction and detail, both of which are required to transform retinal images into recollected constructs.



Long-term changes in spinal cord synaptic strength and perceived pain intensity following high-frequency stimulation (HFS). (A) LTP of C-fiber field potentials in rat spinal dorsal horn following HFS in vivo. After conditioning HFS of the sural nerve (4 x 1s at 100 Hz at 10s intervals), the amplitude of C-fiber evoked field potentials increased up to 100 % above baseline within the first 20 min and remained potentiated throughout the observation period (adapted from Sandkühler and Liu, 1998, n=5). (B) In human subjects, HFS of superficial peptidergic afferents (5 x 1 s at 100 Hz, 10 s interstimulus intervals) induced a longlasting increase of pain ratings to single electrical test pulses through the conditioning electrode by about 50 % compared to a control site (input-specific LTP, n=7). (C) HFS also induced a marked increase of pin prick evoked pain by about 200 % surrounding the conditioned electrode (as compared with an unconditioned control site; heterosynaptic LTP, n=8). Each dot in B and C represents the shift of normalized pain ratings averaged over a five minute time window compared to the unconditioned control site.
(from: Klein et al. Proceedings of the 10th World Congress on Pain, Progress in Pain Research and Management, Vol. 24; IASP Press, Seattle 2003 in press).

Introductory Remarks to Symposium 13

Longterm potentiation and longterm depression of nociceptive CNS processing

Walter Magerl and Rolf-Detlef Treede

Longterm potentiation (LTP) and longterm depression (LTD) of synaptic transmission are well-accepted phenomena of cellular plasticity. Their prominent role in plasticity of the hippocampus and neocortex has prompted the generalization of these neurobiological mechanisms as general models of learning and memory in many species, including human. Due to the lack of convincing evidence of its contribution to acquisition and plasticity of complex behaviours, however, such a role is still disputed. Recent electrophysiological and functional evidence in animals and humans now suggest that LTP- and LTD-like plastic changes are also found in sensory and motor pathways. The symposium is centered around the role of LTP and LTD in the nociceptive system, which has long been known to display prominent plasticity.

The symposium will be opened by T. Bliss, who has first detected the phenomenon of LTP more than 30 years ago. His presentation will focus on development of the concept of LTP in the past decades. A. Artola will then focus on the role of LTP and LTD in hippocampus and visual neocortex, illustrating the central role of intracellular calcium concentration as a mechanism regulating a sliding balance of LTP and LTP. Beyond memory acquisition and consolidation additional mechanisms are imported in long-term storage of memories. In the absence of reinforcement, a resulting behavioural response will gradually diminish to be finally extinct. The importance of extinction, its cellular mechanisms and the role of the endocannabinoid system in extinction of aversive memory is highlighted by W. Zieglgänsberger.

The second half of the symposium is devoted to the role of LTP and LTD in sensory and motor systems. J. Sandkühler will demonstrate that synaptic LTP is a cellular mechanism of central sensitization in the nociceptive system. He will show that LTD and depotentiation can be induced in spinal cord that involve different signal transduction pathways. Eventually, the plasticity of spinal nociceptive processing may be paralled by analogues perceptual changes. W. Magerl will detail input-specific and heterosynaptic functional consequences of LTP- and LTD-inducing stimulus protocols on human pain perception demonstrating the diversity of these mechanisms. The symposium will be closed by U. Ziemann, who will illustrate using the method of transcranial magnetic stimulation that LTP-like plasticity of the human motor cortex is characterized by the principles of input-specificity, cooperativity and associativity. The induction of motor cortex LTP is modulated by dopaminergic, noradrenergic and cholinergic mechanisms.

114 Long-term potentiation after 30 years - where do we stand?

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Abstract has not been submitted.

115 Use-dependent synaptic plasticities in hippocampus and visual cortex

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Long-lasting, activity-dependent changes in synaptic efficacy are likely to underlie fundamental neural processes in the brain, including neural development and information storage. Two opposite forms of activity-dependent synaptic modifications have been identified so far, long-term potentiation (LTP) and long-term depression (LTD). The direction and the degree of the synaptic change are a function of postsynaptic depolarization: low levels of depolarization, for instance in response to low-frequency stimulation of afferent inputs, result in LTD whereas stronger depolarizations, most conveniently produced by high frequency stimulations, lead to LTP. It is long known that synaptic plasticity is finely tuned by factors extrinsic to the synapse to be modified such as activity of converging inputs (GABA-ergic synaptic inhibition, neuromodulators, ...), circulating hormones (e.g. stress-related hormones), ... It is becoming increasingly apparent that the induction of synaptic plasticity is also sensitive to, at least, one other intrinsic factor: previous synaptic activity. Such a modulation is referred to as metaplasticity. The phenomenology of metaplasticity is characterized by concomitant shifts in Θ^-, the threshold for LTD, and Θ^+, the LTD/LTP crossover point as defined by the voltage-response curve for the induction of LTD and LTP, in opposite directions. Θ^- moves progressively toward more polarized membrane potentials (V_{ms}) and Θ^+, conversely, toward more depolarized V_{ms}, as initial synaptic strength increases, opening the voltage window for LTD induction. On the other hand, in depressed synapses, Θ^- is shifted toward more depolarized V_{ms} and Θ^+ toward more polarized V_{ms}, closing the window for LTD induction. Metaplasticity, by facilitating LTD and inhibiting LTP in potentiated synapses and *vice versa* in depressed synapses, thus helps keeping synapses within a dynamic range.

Memory impairments, which occur regularly across species as a result of aging, disease and psychological insults (for example, stress), constitute a useful area for investigation into the neurobiological basis of learning and memory. Streptozotocin (STZ)-induced diabetic rats develop learning deficits. Interestingly, although in STZ-induced diabetic rats hippocampal LTP and LTD can reach the same magnitude as in controls, Θ^- is shifted to more polarized V_{ms} and Θ^+ to more depolarized V_{ms}, thus as in previously potentiated synapses. This suggests that metaplasticity be specifically impaired in STZ-induced diabetic rats. By promoting persistent reductions and preventing persistent

enhancements of synaptic transmission, this should result in a gradual activity-dependent, long-lasting decline in baseline transmission.

Extinction of aversive memory - a role for endocannabinoids? 116

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In the absence of reinforcement, the behavioral response resulting from aversive memories will gradually diminish to be finally extinct. Despite the importance of extinction, its cellular mechanisms are largely unknown. In a recent paper we provided evidence for the involvement of endocannabinoids in the extinction of aversive memories (*Marsicano et al. NATURE, 418. 2002*).

Endocannabinoids are released from neurones in a stimulus-dependent manner. They are cleaved from membrane lipid precursors and are rapidly deactivated by uptake and enzymatic breakdown. Endocannabinoids activate cannabinoid (CB1) receptors on nearby cells or terminals which are molecular targets also for marijuana and hashish. CB1 receptors and endocannabinoids are present in memory-related brain areas and modulate memory by functional interactions between the production of endocannabinoids and various transmitter systems. The natural activators of the CB1 receptor are anandamide, 2-arachidonyl glycerol (arachidonyl ethanol amide) and 2-arachidonyl glyceryl ether.

CB1-deficient mice showed strongly impaired short-term and long-term extinction in auditory fear-conditioning tests, with unaffected memory acquisition and consolidation. Treatment of wild-type mice with the CB1 antagonist SR141716A mimicked the phenotype of CB1-deficient mice, revealing that CB1 is required at the moment of memory extinction. Consistently, tone presentation during extinction trials resulted in elevated levels of endocannabinoids in the basolateral amygdala complex, a region known to control extinction of aversive memories. In the basolateral amygdala, endocannabinoids and CB1 were crucially involved in long-term depression of GABA-mediated inhibitory currents. We propose that endocannabinoids facilitate extinction of aversive memories through their selective inhibitory effects on local inhibitory networks in the amygdala. In addition to memory and cognition these receptors have been involved in euphoria, analgesia, sedation and movement. CB1 activation by agonists or blockade of endocannabinoid inactivation might become a novel target for the treatment of e.g. chronic pain states, which are prototypic examples of aversive memories.

Synaptic LTP and LTD in spinal pathways 117

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Some neurons in the central nervous system not only have the capacity to transmit, inhibit and weigh information, but they may also store information for prolonged pe-

riods of time e.g. by use-dependent changes in synaptic strength. Synaptic plasticity in hippocampus is an extensively studied cellular model of learning and memory (1). Studies *in vitro*, on experimental animals and on human subjects suggest that similar mechanisms also apply to pain pathways and may account for some forms of hyperalgesia, allodynia and analgesia (3,4). Recent studies show that a small but well defined group of neurons in lamina I of spinal dorsal horn play a key role for abnormal pain sensitivity (2, 3).

Repetitive discharges in primary afferent C-fibres induce long-term potentiation of synaptic strength (LTP) between C-fibres and lamina I neurons that express the NK1 receptor (3). Synapses between C-fibres and other nociceptive neurons in lamina I are, in contrast, not affected. This suggests that normal versus abnormal pain sensations are mediated by different ensembles of spinal neurons. LTP induction requires co-activation of NK1 and NMDA receptors and T-Type voltage-gated calcium channels. This leads to a steep rise in free cytosolic calcium ion concentration which in turn activates calcium-dependent enzymes including protein kinase C and calcium-calmodulin-dependent protein kinase II. The kinases phosphorylate synaptic proteins including AMPA receptors, modulate insertion of postsynaptic receptors and may control the phenotype of the neurons. Together these cellular changes synergistically lead to LTP. Interruption of the signal transduction pathways at any site not only blocks LTP induction (3, 4) and but also prevents hyperalgesia and allodynia in behaving animals and human subjects.

The risk that activity in C-fibres induces LTP in nociceptive pathways is substantially higher if endogenous pain control is insufficient or if activity in C-fibres is excessive. Under these circumstances drugs acting at spinal μ-opioid receptors or α_2-adrenoreceptors may not only be antinociceptive but also protective (4).

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(3) Ikeda, H., Heinke, B., Ruscheweyh, R, Sandkühler J. (2003), Synaptic plasticity in spinal lamina I projection neurons that mediate hyperalgesia, Science, 299: 1237-1240
(4) Sandkühler, J. (2000) Learning and memory in pain pathways, Pain 88: 113-118.

118 LTP- and LTD like plasticity of human pain perception

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Strong electrical stimulation of nociceptive C-fiber afferents leads to plasticity of nocicep-tive spinal transmission (LTP or LTD) involving iono-tropic and metabotropic glutamate recep-tors, as well as neurokinin receptors NK1 and NK2 (Randic et al. 1993, Ikeda et al. 2003). LTP at nociceptive spinal synapses was also induced by tissue damaging levels of natural noxious stimulation, which only occurred in spinalized, but not spinal cord-intact animals indicating suppression of LTP induction in animals with intact neuraxis by descending supra-spinal control systems. Interest-ingly, conditioning high-frequency electri-cal stimula-tion of C-fibers was able to overcome descending inhibitory controls allowing LTP induction also in intact animals.

The role of LTP as a mechanism of plasticity in human perception, however, was until re-cently com-pletely unknown. Several lines of arguments, however, suggested that some forms of hyperalgesia (secondary hyperalgesia) may represent perceptual correlates of heterosynaptic LTP of pain perception (Treede and Magerl 1995, Magerl et al. 2001). Studying changes of human pain perception using those conditioning stimulus protocols, which reliably elicited LTP and LTD in intact animals, we found evidence for homo- and heterosynaptic LTP- and LTD-like plasticity of human pain perception (Klein et al. 2003). LTP of human pain perception involved NMDA-dependent and independent subtypes. This supports the assumption that multiple forms of nociceptive LTP may also exist in humans, and have a role in human pain perception.

Klein T, Magerl W, Mantzke U, Hopf HC, Sandkühler J, Treede RD (2003) Long-term poten-tia-tion of human pain perception. In: JO Dostrovsky, DB Carr, M Koltzen-burg (Eds.) Progress in Pain Research and Management, Vol. 24, Proceedings of the 10th World Con-gress on Pain, San Diego 2002, IASP Press, Seattle (in press).

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LTP-like plasticity in intact human motor cortex. Investigations with transcranial magnetic stimulation. 119

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Long-term potentiation (LTP) refers to a persistent increase in the size of the synaptic component of the evoked response, recorded from individual neurones or from populations of neurones, typically in slice preparations of the brain. Most often, LTP is induced by repeated stimulation of the pathway of interest. Characteristic properties of LTP are cooperativity, associativity and input-specificity. Furthermore, many forms of LTP depend on NMDA receptor activation.

Is it possible to induce and measure LTP in intact humans? Repetitive transcranial magnetic stimulation (RTMS) of the motor cortex may induce a persistent (> 60 min) increase in motor cortex excitability as revealed by an increase in motor evoked potential (MEP) amplitude. This effect takes place only if the motor cortex is disinhibited at the time of RTMS. Disinhibition was produced by a transient ischemic limb nerve block which results in a rapid decrease of GABA, the major inhibitory neurotransmitter, in the contralateral sensori-motor cortex (Levy et al. 2002, Ann Neurol 52: 755-61). RTMS (0.1 Hz) alone did not affect MEP size, ischemic nerve block alone led to a mild and short-lasting MEP increase, whereas RTMS in the presence of ischemic nerve block resulted in a strong and long-lasting (> 60 min) MEP increase (Ziemann et al. 1998, J Neurosci 18: 1115-23). Therefore, cortical disinhibition lowered the threshold for induction of the persistent MEP increase (cooperativity). Furthermore, the MEP increase was observed only if the motor representation of interest was effectively stimulated by RTMS (input-specificity) (Ziemann et al. 2002, J Neurosci 22: 5563-71). It is possible to

induce the MEP increase in the absence of disinhibition, if RTMS is paired with another stimulus. This was done by pairing each RTMS pulse with electrical stimulation of the median nerve at the contralateral wrist. The MEP increase induced by this associative stimulation was critically dependent on the inter-stimulus interval between the two stimuli and occurred only when the median nerve stimulus preceded the RTMS pulse by about 25 ms (associativity) (Stefan et al. 2000, Brain 123: 572-84). The persistent increase in MEP size induced by these cooperative or associative RTMS protocols was blocked by pre-treatment with the NMDA blocker dextromethorphan (Ziemann et al. 1998, J Neurosci 18: 7000-7; Stefan et al. 2002, J Physiol 543: 699-708).

Findings suggest that characteristic properties of the RTMS induced persistent MEP increase in intact humans are cooperativity, associativity, input-specificity and dependence on NMDA receptor activation. This raises the hypothesis that similar mechanisms underlie the persistent MEP increase and LTP.

122 Selective induction of Homer1a in spinal neurons during pathological pain states via activation of NMDA receptors and Erk1/2

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The metabotropic glutamate receptors mGluR1/5, have gained importance as modulators of activity of both peripheral nociceptors as well as of spinal nociceptive neurons they synapse on. Although it is known that mGluR1/5 contribute significantly to synaptic plasticity in the spinal cord leading up to clinical manifestations of chronic pain, the underlying molecular signalling events are still unclear. Proteins of the Homer family bind mGluR1/5, inositol-3-phosphate receptors (IP3R) and Shank family proteins and are involved in signal transduction and spatial targeting of mGluR1/5 in dendritic spines. The present study describes the localization of Homer1 proteins in pain pathways and its regulation in pathological pain states in rats. Our findings demonstrate that Homer1b/c are strongly and constitutively expressed in peripheral and spinal neurons involved in nociception, where they likely contribute to the targeting of mGluR1/5 -IP3R signalling complexes at nociceptor terminals. In contrast, Homer1a is dynamically regulated by persistent nociceptive activity selectively in dendrites of spinal nociceptive neurons, concurrent to the development of nociceptive hypersensitivity. Moreover, we elucidate here the signalling mechanisms underlying activity-induced upregulation of Homer1a *in vivo* in the context of peripheral inflammation, a pathological stimulus encountered frequently in clinical states. Induction of the Homer1 gene in nociceptive neurons by peripheral inflammation depends upon the activation of spinal NMDA receptors and extracellular signal-related kinase/ MAP kinase and is modulated by the protein kinase Src. Thus, induction of Homer1a may represent an additional mechanism via which chronic activation of nociceptors leads to long-lasting changes in efficacy at spinal nociceptive synapses.

Changes in Neuronal Calcium Signalling During Diabetic Pathology

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Diabetes mellitus is one of the most common illness among known human pathologies. There are different aspects of its development. Neuronal pathology connected with possible development of diabetic neuropathy is among them. There are different mechanisms that can lead to changes in neuronal function.. Changes in intracellular calcium metabolism and calcium signaling were shown on many nonexcitable insulin-sensitive cells. To investigate the possible changes of calcium signaling in neuronal cells possibly connected with different types of neuropathy investigations of calcium intracellular metabolism in peripheral and central neurons were made in connection with their response to nociceptive and nonnociceptive stimuli. In our previous investigations we found an elevation of residual calcium in nociceptive neurons under the case of hyperglycemia; therefore we tried to answer the question what is the reason for such changes. It is of interest also was why we meet such changes in only one type of neurons. Changes in Ca exchange in mitochondria which can be connected with different changes in calcium exchangers or calcium uniporter were found, as well as changes in Ca accumulation by the endoplasmic reticulum in neurons of different levels. Besides this changes in the action of Ca channels antagonists were observed. At the same time no changes in the amplitude or density of calcium currents have been shown. The close connection between changes in different types of intracellular calcium structures gives the possibility to think about their main role in early changes in calcium signaling under the state of hyperglycemia and other metabolic changes characteristic for diabetes mellitus. They can be the reason for early alterations in the transduction of sensory signals between peripheral and central neurons and the development of pain syndromes.

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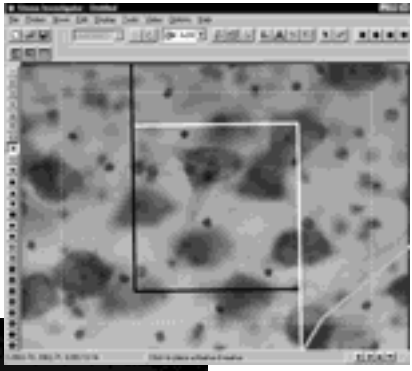

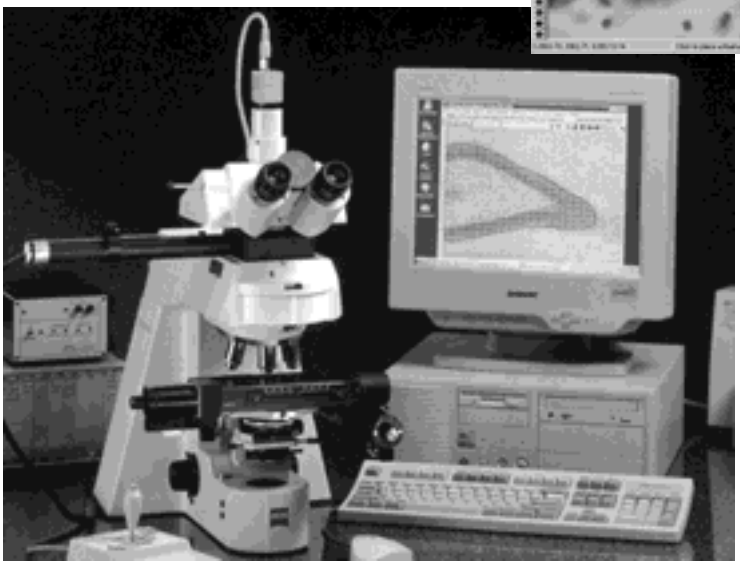

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Introductory Remarks to Symposium 14

Towards a molecular understanding of behavior

Ralf Heinrich and Edward A. Kravitz

Behavior is generated by the functional interplay of neurons within and between neural networks. The contribution of an individual neuron to the generation of a particular behavior is determined by the currently expressed molecular machinery (transmitters, receptors, channels, second messengers etc.) involved in transforming incoming stimuli into characteristically patterned electrical activity. The responsiveness of a neuron or a neural circuit is not fixed; rather it is dynamically modulated by previous activity to produce adaptive changes in the occurrence and performance of a particular behavior. Alterations in neural activity may persist for periods ranging from seconds to lifetime of an organism and may result from reversible modulation of intracellular signaling pathways, altered gene expression or changes in morphology and synaptic coupling.

This symposium presents studies using both invertebrate and vertebrate models in which recent progress has allowed investigators to directly link molecular mechanisms, neural functions and the control of specific behaviors.

Two presentations deal with long-term changes in central pattern generating circuits. Ron Harris-Warrick describes a homeostatic mechanism in crustacean stomatogastric ganglion neurons that compensates for the overexpression of a potassium channel encoded by the shal gene by upregulation of a hyperpolarization-activated inward current. The consequences of this change are that the firing properties of the neurons are not significantly changed. David Parker and Sarah Bevan demonstrate multiple effects of the neuropeptide substance P on second messenger pathways, RNA- and protein synthesis and ultrastructural changes in synaptic terminals. These cause characteristic changes in the activity of the lamprey spinal cord locomotor network.

The next two contributions deal with the functional analysis of second messenger pathways in insect brains. Ralf Heinrich focuses on the role of these pathways in specific arousal and the selection of situation-specific acoustic communication patterns in grasshoppers. Uli Müller describes, how a characteristic temporal activation of different signaling pathways contributes to distinct features of memory formation during associative learning of honeybees.

Social behaviors are considered to be controlled by multiple internal and external signals. Edward Kravitz introduces *Drosophila melanogaster* as a genetically accessible preparation for studies on the central nervous control of aggression. Conditions have been defined in which fighting behavior is routinely seen between pairs of male flies, the behavior has been quantitatively analyzed, and through the use of the GAL4/UAS system driving the expression of a temperature sensitive form of the protein dynamin, individual amine neuron types can be switched on and off while animals are fighting to observe effects on the behavior. In a comparative study on monogamous and nonmonogamous voles, Larry Young identified differences in a specific repetitive sequence in the promotor of the gene coding for the V1a vasopressin receptor. The presence or absence of this sequence determines both the expression pattern of the V1a receptor in the brain and the ability to form pair bonds following mating-induced release of arginine vasopressin in the central nervous system.

124 Potassium channels and the control of a rhythmic behavior

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The pyloric network in the lobster stomatogastric ganglion is subject to extensive neuromodulation to reconfigure its motor pattern over time. Dopamine can initiate a rhythmic motor pattern and can alter all of the parameters of an ongoing pyloric motor pattern. It does so by modulating the strength of synaptic interactions and the activity of voltage-dependent ionic currents that shape the intrinsic firing properties of the component neurons. Dopamine modulates the transient potassium current, I_A, in nearly all the cells, although in different ways to excite or inhibit the cells. The *shal* gene encodes I_A in these neurons. We attempted to modify the firing of pyloric neurons by injecting *shal* RNA; this dramatically increased I_A but surprisingly did not change the neuronal firing pattern at all. Further study showed that the neurons activate a homeostatic mechanism involving upregulation of the hyperpolarization-activated inward current, I_h, to compensate for the increase in I_A. There is a steep linear relationship between the RNA-induced increase in I_A and the neuron?s homeostatic increase in I_h. This response did not depend on changes in firing of the neurons, since a mutant, nonfunctional *shal*RNA could also upregulate I_h. Such compensatory mechanisms could help to protect neurons against over-modulation, and maintain the pyloric firing pattern within an acceptable range of activity in the face of changing modulatory conditions.

125 Cellular and synaptic effects contributing to long-term neuropeptide-mediated modulation of a spinal cord locomotor network.

David Parker and Sarah Bevan

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To understand the neural basis of behaviour, identified cellular, synaptic, and molecular mechanisms must be examined in the neural networks that underlie specific behaviours. In the lamprey, a lower vertebrate, the properties of identified neurons in the locomotor network can be examined in the intact spinal cord *in vitro*. This allows an integrated analysis of the mechanisms underlying network activity and its plasticity.

A single application of the neuropeptide substance P evokes short or long-term (>24 h) concentration-dependent modulation of the frequency and regularity of ongoing locomotor network activity. These effects have different underlying mechanisms, suggesting that they reflect distinct effects of substance P on the network.

The burst frequency modulation has three phases: an induction phase (~2 h) that is associated with the protein kinase C-mediated potentiation of NMDA receptors; an intermediate phase (~2-15 h) that requires protein synthesis, but not de novo RNA synthesis; and a final phase (>15-20 h) that requires de novo RNA synthesis. The maintenance phase is associated with the modulation of the activity-dependent plasticity of glutamatergic inputs from excitatory network interneurons, an example of metaplastici-

ty. The synaptic metaplasticity shares the same induction and maintenance mechanisms as the burst frequency modulation, suggesting that these two effects are causally related.

In contrast to the burst frequency modulation, the effect of substance P on the burst regularity is protein kinase A-dependent. Its maintenance is associated with PKA-dependent changes in the regularity of synaptic inputs during repetitive spike bursts, and also in changes in the regularity of presynaptic spiking.

The effects of substance P on the locomotor network are associated with ultrastructural changes in spinal synapses. These changes include an increase in the number of docked vesicles and changes in synaptic structure.

These results suggest that protein synthesis-dependent changes in the properties and structure of network synapses contribute to the long-term modulation of the locomotor network. The goal now is to identify how specific molecular changes contribute to the cellular and synaptic effects underlying network activity and its plasticity.

Supported by the Royal Society, the Biotechnology and Biological Sciences Research Council, and the Newton Trust.

Selection and control of behavior by intracellular signaling pathways in the insect brain

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Acridid grasshoppers communicate with specific song patterns in the context of attracting partners for reproduction, courting and agonistic behavior. When to sing and which song pattern to perform is determined by the brain, particularly by the central body complex and a distinct neuropil that contains the dendrites of command neurons, each of which controls the performance of a particular stridulatory pattern. Sensory information related to acoustic communication behavior is first analyzed by specific neural circuits and then relayed to the above mentioned central areas of the brain to generate arousal that promotes the production of specific sound patterns. Both, the functional role and the cellular basis of this arousal, have been the subjects of our recent studies.

Arousal is mediated by activation of intracellular signaling pathways connected to G protein-coupled receptors such as the muscarinic acetylcholine receptor (mAChR). The second messenger pathways were characterized by injections of membrane permeable drugs known to interfere with specific steps of intracellular signaling cascades. Muscarinic AChRs in the grasshopper brain mediate excitation by activation of both, the adenylyl cyclase / cAMP / protein kinase A pathway and the phospholipase C / inositol trisphosphate (IP3) / diacylglycerine pathway. Since forskolin-stimulated stridulatory activity could be suppressed by neomycin, which prevents the formation of IP3, the generation of excitation seems to involve a sequential activation of (1st) adenylyl cyclase- and (2nd) phospholipase C- initiated mechanisms. Functionally, the status of activation of these signaling pathways connected to mAChRs in the cephalic control centers underlies specific arousal that promotes the release of song production. However, if stridulation was stimulated by a single injection of ACh into the cephalic control regi-

ons, excitation seemed to be exclusively mediated by nicotinic AChRs. A contribution of mAChR, leading to longer periods of stridulatory activity, only appeared with repeated ACh stimulation at sufficiently short intervals. Male courtship consists of numerous separate song sequences intended to increase both male arousal and the female's readiness for mating. During prolonged courtship, second messengers leading to increasing arousal slowly accumulate in the control circuits for male courtship stridulation and induce the selection of additional song patterns associated with higher courting intensities. Eventually, high arousal initiates an attempt to copulate with the courted female. Courtship stridulation with gradual inclusion of song patterns associated with the progress of courting could also be stimulated by a single injection of the muscarinic agonist pilocarpine. This suggests that the level of second messenger-mediated arousal in the cephalic control regions not only modulates the general threshold to initiate stridulation but also determines the selection of song patterns according to the situation encountered.

127 Second messenger cascades: Major mediators of memory formation

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Memory exists in a variety of temporal domains ranging from short-term memories in the range of minutes to long-term memories (LTM) lasting a lifetime. Evidence from organisms as diverse as molluscs, insects, birds and mammals shows that distinct molecular mechanisms contribute to the multiphasic process of memory formation. Although it is a general characteristic that the sequence or the temporal succession of the stimuli during training is critical for induction of a particular memory, little is known about how these training features modulate the molecular processes and how this affects memory formation. Associative olfactory learning in honeybees provides a perfect system for studying the highly dynamic modulations of molecular processes during the training procedure and the processes of memory formation. Our analysis revealed that the second messenger cascades involving protein kinase A (PKA) and protein kinase C (PKC) partake in different processes during learning and memory formation. We demonstrate a tight connection between the training procedure, the temporal activation pattern of the cAMP/PKA cascade and the induction of olfactory LTM. Only a training procedure that induces a distinct PKA activation pattern in the range of minutes leads to a LTM. While this temporally defined activation of the PKA during training is critical for LTM, the proteolytic cleavage of the PKC during the training procedure leads to an autonomous PKM that is required for the maintenance of a mid-term memory in the range of hours.

Supported by the Deutsche Forschungsgemeinschaft

Fighting Fruit Flies: A model system for the study of aggression

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Our previous studies centered on a crustacean model of aggression using socially naive juvenile lobsters to examine the behavior and to determine the consequences within the nervous system of winning and losing fights. While lobsters are excellent animals for such studies, serious limitations of the lobster system surround the inability to utilize genetic methods to examine the behavior. The fruit fly, *Drosophila melanogaster*, offers those possibilities in an animal that is readily available, easy to maintain in the laboratory, and that shows agonistic behavior between pairs of male flies under appropriate experimental circumstances.

Although not widely known, *D. melanogaster* males do fight, and early studies analyzed the behavior and defined many of its components (Hoffmann, Anim. Behav. 35: 807-818, 1987). We have simplified the experimental set-up used by earlier authors to the point where only two male flies are in the fighting chamber, along with a headless mated female to attract the males to the surface of a small food cup that is illuminated by an overhead light (Chen et al., Proc. Natl. Acad. Sci. 99: 5664-5668, 2002). Using this chamber 73 fights were carried out involving over 2000 encounters (interactions) between the flies and 9000 behavioral transitions to construct an ethogram, perform descriptive statistics and carry out a Markov Chain Analysis of the behavior. In an average 30 minute paired trial, we observed 27 encounters between the flies lasting a mean of 11 seconds duration and showing various intensity levels. The chain analysis demonstrated that flies spend a very small percentage of the time at the highest intensity levels (boxing and tussling).

With the behavioral analysis completed we have begun studies using the GAL4/UAS system to regulate amine neuron function in flies during agonistic encounters. We have obtained GAL4 lines expressing the yeast transcription factor under the control of: (i) a DOPA decarboxylase enhancer (courtesy of J. Hirsh) allowing selective expression in dopamine (DA) and serotonin neurons; and (ii) a tyrosine hydroxylase enhancer (courtesy of S. Birman) allowing selective expression in DA neurons. In addition we have generated a transgenic tyramine-β-hydroxylase-GAL4 line allowing selective expression in octopamine neurons. These lines are crossed with a UAS line driving the expression of a temperature sensitive mutant form of the protein dynamin. In the progeny of the cross, at the permissive temperature (25^{o}C) amine neurons expressing the mutant form of dynamin should function normally, while at the restrictive temperature (30^{o}C), vesicle recycling is blocked. Thus a temperature jump should cause a selective, rapid and reversible cessation of transmitter release in amine neurons containing the mutant protein. Preliminary results show a reduced number of encounters at 30^{o} in animals in which DA neuron function is influenced in this way. Mutant flies are capable of movement at both temperatures and encounters that do take place appear normal (supported by NIGMS and NSF).

129 Vasopressin and Social Attachment in a Monogamous Mammal

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Voles, genus *Microtus*, provide an excellent opportunity to investigate the molecular, cellular and neurobiological mechanisms underlying complex social behaviours. Prairie voles are highly gregarious, biparental and monogamous. Upon mating, prairie voles form enduring social attachments with their mates. Pharmacological studies have demonstrated that the neuropeptide arginine vasopressin (AVP) is the molecular trigger that stimulates the formation of the pair bond in the male for his female partner. In contrast to the prairie vole, the genetically closely related montane vole is rather asocial, promiscuous, and does not form social attachments. Comparisons of the AVP systems at a neuroanatomical and genetic level in prairie and montane voles have provided insights into the molecular basis of monogamy in voles. Although the distribution and expression of the AVP peptide is similar between the species, the distribution of the AVP V1a receptor (V1aR) is strikingly different between prairie and montane voles. Of particular interest, the V1aR is concentrated in the ventral pallidum, a component of the mesolimbic reward pathway, in prairie voles but not in montane voles. Infusion of V1aR antagonists into this region prevents partner preference formation in male prairie voles, and increasing V1aR expression in the ventral pallidum using viral vector gene transfer facilitates partner preference formation. A survey of other mammalian species pairs suggests that this pattern of V1aR expression is associated with monogamy. Thus species differences in V1aR gene expression in the reward pathways likely account for the species differences in social attachment. Comparisons of the V1aR gene in these species demonstrate that while the coding regions are highly conserved, the promoter of the prairie vole V1aR gene contains a 430 bp sequence of repetitive, or microsatellite DNA, approximately 700 bp upstream of the transcription start site which could account for the species differences in expression pattern. Gene transcription assays using reporter gene constructs support the hypothesis that mutations in this region of the promoter can have a profound effect on expression of the gene, and therefore potentially explain the species differences in social behaviour and provide a molecular mechanism for the evolution of monogamy.

131 The potency of acetylcholine to activate muscarinic receptors in the brain of grasshoppers

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The species- and situation-specific acoustic communication behavior of acridid grasshoppers can be stimulated by experimental activation of cholinergic synaptic pathways in the central body complex and a small brain neuropil where command neurons that control the performance of particular song patterns extend their dendrites. Both, nicoti-

nic and muscarinic receptors, contribute to the excitation of the cephalic control system and the initiation of sound production. Stimulation of the above mentioned brain areas by pressure injection of ACh, the presumed natural transmitter, elicits short-latency and short-duration stridulatory activity. These characteristics are similar to nicotine-stimulated stridulation but lack the features of muscarine-stimulated behavior, suggesting that muscarinic receptors are not effectively stimulated by ACh. In contrast, song patterns of higher complexity and song patterns that only appear after prolonged courtship seem to require muscarinic ACh receptor-mediated arousal of the cephalic control system. The following studies attempted to resolve this mismatch.

Periodic injections of ACh with inter-stimulus intervalls of 15 and 30 seconds elicited stridulatory activity of progressively increasing duration (from less than 1 to 5 seconds). This increase was significantly reduced or absent when the muscarinic receptor antagonist scopolamine was co-injected with ACh or when inter-stimulus intervals exceeded 30-45 seconds, suggesting that muscarinic receptor-initiated signaling pathways are weakly activated by single ACh applications but can cause accumulating excitation with sufficiently high stimulus repetition rates. When stridulation was stimulated by repeated injections of muscarine, the overall duration of stridulatory activity was prolonged and cumulative effects from one stimulus to the next could be detected with inter-stimulus intervals of up to 10 minutes. This indicates a prolonged presence of muscarine at the receptors and/or a stronger and more persistent activation of the second messenger pathway coupled to the muscarinic receptors in this brain region. A factor that may limit the potency of ACh to effectively stimulate muscarinic receptor-mediated excitation could be the ACh-esterase that inactivates synaptically released ACh. ACh-esterase was detected by a histochemical procedure and found to be present in the cephalic regions that control stridulatory behavior in grasshoppers. Co-injections of ACh with the ACh-esterase inhibitor eserine lead to a reversible increase of stridulatory activity with low concentrations of esterase inhibitors but to a reversible suppression of ACh-inducible stridulation at higher concentrations. The functional role of muscarinic receptor-induced arousal for the promotion of stridulatory behavior was further studied by attempts to increase the effect of sensory stimuli relevant to grasshopper acoustic communication behavior through inhibition of ACh-esterase activity in the cephalic control neuropils.

Grasshopper acoustic communication behavior is inhibited by activation of the NO-/cGMP- signaling pathway in the brain **132**

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Grasshoppers perform acoustic communication behavior in the context of mate finding, courtship and rivalry. While the neuromuscular excitation patterns underlying sound production are generated by thoracic circuits, the decision about when and which song pattern to sing is made by the brain, in particular by the central body complex and a neuropil where the stridulatory command neurons have their dendrites. Stimulation of muscarinic ACh receptors in these brain areas has been demonstrated to activate adeny-

late cyclase- and phospholipase C- signaling pathways leading to excitation that promotes song production.

Accumulation of cyclic GMP in neurons of the above mentioned brain areas inhibits muscarine-stimulated stridulation. Injections of either 8-Br-cGMP, a membrane permeable analogue of endogenously generated cGMP, or of zaprinast, an inhibitor of cGMP-specific phosphodiesterase, caused a significant and reversible reduction of stridulatory activity. A similar inhibitory effect on muscarine-stimulated stridulation was achieved by focal application of fresh solutions of the NO- (nitric oxide-)donor sodium nitroprusside. In contrast, old and inactive solutions of sodium nitroprusside or co-injections of sodium nitroprusside with ODQ, a membrane permeable inhibitor of soluble guanylate cyclase, had no effect on the duration of muscarine-stimulated singing behavior.

To identify putative endogenous sources of NO, the NADPH-diaphorase staining procedure was applied to vibratome sections of grasshopper brain. Among other areas described by earlier studies, the central body, particularly its lower division and one layer of its upper division, was intensely stained. This suggests that neurons of the central body complex may express nitric oxide synthase and generate NO to inhibit the performance of stridulatory behavior. Following incubation with both, zaprinast and sodium nitroprusside, isolated grasshopper brains were rapidly fixed, embedded and sectioned. Neurons that responded to these conditions with increased levels of cytososlic cGMP were labeled with antibodies raised against this second messenger molecule. Within the entire brain, only two rostrally and ventrally situated groups, each consisting of approximately 75 cell bodies were intensely stained. Their labeled axons projected as tracts through the entire brain, with few axons finally entering a peripheral nerve that originated from the tritocerebrum. Incubation of brains with zaprinast in the absence of NO resulted in weak labeling of the same neurons. Saline incubated brain showed no detectable staining of cGMP. Whether and which neurons of the NO-responsive cell groups contribute to the control of grasshopper stridulatory behavior will be determined by focal release of NO in those brain regions where its inhibitory effect can be observed.

133 Fight or Flight? Octopamine Effects on the Cricket Escape Pathway

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Escape behavior in crickets not only is crucial for individual survival but also plays an important role in conspecific aggression: During aggressive encounters escape behavior marks both the end and the final decision of a cricket fight since only the eventual 'loser' shows escape running. Triggering of escape behavior is directly controlled by the spike activity of the sensory-to-motor giant interneuron (GIN) pathway that is activated by mechano-sensory afferents coupled to the abdominal cercal sense organs. Thus, the sensitivity of this network could be crucial for the decision making process in the CNS during aggressive interactions. Electrophysiological, pharmacological and behavioral

studies in crickets show that biogenic amines such as octopamine (OA), dopamine or serotonin may affect synaptic transmission, sensory sensitivity and activation thresholds [Goldstein, Camhi J Comp Physiol A 168,103,1991; Hörner et al. Verh Dtsch Zool Ges: 90,10,1997; Stevenson et al. J Neurobiol 43,107,2000; Heblich, Hörner Proc. 28th Göttingen Neurobiol Conf 758,2001]. Immuncytochemistry revealed endings from amine-containing neurons in the region of monosynaptic connections between wind-sensitive afferents and post-synaptic giant interneurons in the terminal ganglion [Spörhase-Eichmann et al. Cell Tiss Res 268,287,1992; Hörner et al. ibid. 280,563,1995]. The present study describes amine-induced changes of electrical properties in pre-/postsynaptic neurons and how modulation of cellular and network characteristics may ultimately influence behavioral choice.

Electrophysiological recordings were made from identified postsynaptic GINs, presynaptic afferents and other elements of the pathway both in-vivo and in-vitro. The effects of bath-applied aminergic drugs were tested during spontaneous or stimulus-evoked activity.

In-vivo recordings from GINs demonstrate that OA, the analogue of norepinephrin in invertebrates, slightly depolarizes the resting potential, increases the membrane resistance and the frequency and amplitude of spontaneous PSPs, which leads to spontaneous spiking normally never occurring in GINs without stimulation. Furthermore, OA enhances the GINs' spike response to synaptic activation after electrical stimulation of the cercal nerve by lowering their spike threshold. However, despite this enhanced excitability of GINs, the stimulus response of sensory neurons (i.e. the number of spikes/stimulus) remains unchanged. These findings suggest that OA either acts on interneurons presynaptic to the GINs or affects postsynaptic GINs directly. The finding that OA induces 'spike-broadening' in postsynaptic GINs supports the latter hypothesis. Both, the delayed repolarization phase of action potentials and the increased membrane resistance point to direct OA-induced changes of potassium conductances in GINs. In-vitro recordings from isolated GIN cell bodies, which show reduced 'delayed-rectifier' potassium currents upon OA application, provide further evidence for this. However, OA effects on other neuron types have also been found. OA effects can be blocked by epinastine, an antagonist of the neuronal OA3 receptor.

The described OA-induced changes of membrane properties and stimulus response levels suggest direct postsynaptic OA actions on GINs. Thus, an OA-induced modulation of potassium currents found in GINs could be one mechanism generating an increased excitability level with lowered spike thresholds, which may contribute to a more sensitive escape reaction with lowered behavioral thresholds observed upon OA application.

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134 The tyramine receptor of *Caenorhabditis elegans*

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The biogenic monoamines octopamine and tyramine control various behaviours in the soil nematode *Caenorhabditis elegans*. Among the behaviours that are modulated by these compounds are egg-laying, defecation and movement. The most pronounced effects of octopamine and tyramine can be seen in locomotion. Whereas octopamine and tyramine induce hyperactivity in the worms, treatment with yohimbine, a tyramine receptor antagonist, results in lethargic worms. This effect is dose-dependent, which indicates specific interaction with a target molecule. Gene-silencing experiments with all putative octopamine/tyramine receptors revealed the C02D4.2 receptor as the one that transmits the known effects of these compounds. Although the transmitter is present in only two neurons, several neurons, including many motor neurons, and intestinal muscles express the receptor molecule. A phenotypic analysis revealed that gene-silencing of the corresponding receptor gave almost the identical phenotype as yohimbine treatment, which is also identical with the phenotype of worms defective in octopamine synthesis. In addition to the effects seen under yohimbine treatment it appears that modulation of the locomotory activity is organized in a hierarchical way. Serotonin- and dopamine- mediated fine-tuning of locomotory behaviour occurring in well-fed and hungry worms is overruled by the presence or absence of octopaminergic signalling. Heterologous expression of the receptor molecule revealed a tyramine receptor identity. Evaluation of the intracellular signalling pathway revealed a coupling to the Gqalpha-protein. Other Galpha-proteins are not involved in this pathway. Consequently, phospholipase C is likely to be further downstream the octopaminergic signalling. And phospholipase-knockout worms actually show the expected phenotype. Downstream the phospholipase C we observed an interesting dichotomy that might be the molecular switch that combines behavioural and physiological effects. Gene silencing as well as yohimbine treatment revealed another interesting phenotype, the development of obesity. These worms deposit fat droplets in and outside the intestine.The tyramine depleted worm is one of the most intriguing models to study the molecular basis underlying obesity and type II diabetes, both among the most prevalent diseases in western countries. (This work was supported by grants of the DFG (R0 1241)).

135 To Mate or to Fight? Effects of Flight on Male-Female Relationships in Cricket *Gryllus bimaculatus*

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In *G. bimaculatus*, flight has been shown to enhance aggressive encounters between males [1,2]. Here, we demonstrate that, in this insect, flight affects male-female relationships as well.

The specimens, 2 –3 weeks after imaginal moult, taken from a breeding colony (28oC, 13h-11h light-dark cycle), were kept isolated with food and water in abundance for 24 h before the experiment. To evoke flight, an animal was glued to a holder and suspended in an air stream. After 3-min flight, the pair members were placed into a transparent arena (20x12x12 cm). Interaction was allowed 5 min later by removing a separating wall. Behaviours were observed for 5 min since the first physical contact between the animals.

We found that flown males show enhanced courtship and significant success in copulation. The calling and/or courtship song was produced by 15 of 16 flown males (ca. 94%). In contrast, only 6 of 24 non-flown control males (25%) were singing while meeting a female ($F = 24.2$, $p < 0.001$). In 8 of 16 pairs with flown males, the courtship behaviour resulted in copulation (50 %) versus nil in the control ($F = 23.7$, $p< 0.001$). Flight effect on male courtship was also observed in pairs with flown females. Calling and/or courtship songs were produced by 12 of 17 (72.2 %) of flown males versus 11 of 30 (36.7 %) of non-flown males ($F= 6$, $p < 0.05$). In that case, however, elevated courtship was not followed by significantly increased copulation (16.7% vs. 10%, $F = 0.44$, NS). We believe that flight-induced aggressiveness of females was the cause for lower occurrence of copulation in the group containing both animals flown in comparison to that in pairs with only male flown. Indeed, in the former group, fighting occurred in 72% of pairs while in the latter group, in 13% pairs, the mean duration of fighting was 10.4 and 2.2 sec, respectively ($F = 17.6$, $p< 0.001$).

These findings extend the original observation that flight evokes reset of aggressiveness in fight losers[1]. With respect to male crickets, it appears that all so far discovered effects of flight, including the inhibitory one on escape behaviour[2], are in fact various manifestations of a single change, namely, dominance promotion. A mechanism thus exists that directly, apart from male-male encounter, awards the higher social rank to a specimen that has performed a motor action providing territorial expansion. It is likely that the neuromodulator octopamine plays a role in this mechanism, as the natural release of octopamine accompanies flight[3], and the octopamine agonist chlordimeform mimics the flight induced reset of aggressiveness in fight losers[2].

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Functional dissection of the octopaminergic neurotransmitter system in ethanol tolerance in *Drosophila* 136

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Tolerance is a common phenomenon associated with chronic drug abuse and it is partially mediated by adaptations of the brain. Tolerance develops upon repeated ethanol exposure and can be observed on the behavioral level as an increase of resistant to the same ethanol dose after previous exposure. To identify the molecular genetic and neuro-

nal basis underlying these adaptive processes we established *Drosophila melanogaster* as a model system. Wild-type flies develop tolerance to the effect of ethanol on postural control and sedation after a previous exposure to ethanol vapor. These behavioral changes require the functional and structural integrity of the brain as determine by targeted expression of tetanus toxin and the analysis of EMS-induced mutants carrying lesions in the brain. The behavioral and phenotypical analyses of different behavioral mutants demonstrated that at least two different mechanisms contribute to tolerance. One mechanism is revealed by the analysis of a mutant with defects in the cellular stress response and suggests that tolerance based in part on cellular integrity. The second mechanism is uncovered by the *Drosophila* tyramine-β-hydroyxlase (TbetaH) mutant unable to synthesize the neurotransmitter, Octopamine. Octopamine is thought to be the functional analogue of noradrenaline in insects and play important role in a wide variety of behaviors, like reinforcement in the honey bee or learning and memory in *Drosophila*. The discovery of a function for Octopamine in ethanol tolerance enables us now to dissect the different behavioral components and their contributions to the development of tolerance.

Introductory Remarks to Symposium 15

Peptide co-transmitters in identified neurons

Petra Skiebe and Sabine Kreissl

The function of the nervous system not only depends on the connectivity of the neuronal networks, but also on the transmitters released by the neurons. Single neurons contain not one, but multiple transmitters, which often included neuropeptides. The number of isolated neuropeptides is large (more than one hundred) compared with the number of classical transmitters, and it is therefore necessary to study the effect caused by the release of a transmitter 'cocktail', in order to understand the plasticity of the brain. To investigate the role of peptide co-transmission, it is advantageous to work on identified neurons with known peptidergic co-transmitters, known function and known targets. These prerequisites are met in some crustacean systems in which peptides can be investigated on the level of single identified neurons using a combination of biochemical, anatomical and electrophysiological methods and by modeling.

Peptides are isolated by biochemical methods and their distribution can then be investigated by immunocytochemical methods in order to get a first idea concerning their function. Combining anatomical with genetic methods shows that a differential distribution of peptides can be due to a different distribution of precursors, resulting in putatively diverse physiological actions (Heiner Dircksen). The co-transmitters not only vary between neurons but also in a given neuron depending on the developmental stage (Valèrie Fénelon). Motoneurons, for example, transiently express FMRFamide-like peptides, suggesting a possible role in the establishment of the mature neuromuscular junction. Furthermore, different modulatory projecting neurons acquire FMRFamide-like immunoreactivity at different developmental stages, indicating different functional roles during development. Using only immunocytochemistry to identify peptides present in a given neuron can be misleading, since the antibody might recognize different peptides or different members of the same family. By combining immunocytochemistry with MALDI-TOF (Matrix-assisted laser desorption time-of-flight) mass spectrometry, it is possible to identify particular peptides or even multiple members of a peptide family in single identified neurons, which is necessary in order to investigate co-transmission or the role of different family members (Petra Skiebe).

One good model system to investigate the function of peptides is the neuromuscular junction. Sabine Kreissl and co-workers show that two peptides elicit antagonistic effects on muscles. This is interesting because they are likely to be co-released either as transmitters from a pair of identified motoneurons or as hormones from the pericardial organ, a major neurohaemal organ in crustaceans. That the co-transmitters of a particular modulatory interneuron have different effects depending on the circuit they are influencing has been shown using the stomatogastric nervous system (Wolgang Stein). These co-transmitters have a converging action in one circuit, while a diverging action on the second. Combining dynamic clamp experiments and modeling, the mechanisms through which the peptide Red Pigment-Concentrating Hormone (RPCH) shape the output of a network of identified neurons by acting on multiple cellular and synaptic targets have been analyzed (Prinz). RPCH has three different effects, and due to this combination of methods it is possible to judge the contribution of each of the effects on the output of the neural network.

137 Differential distributions and functions of orcokinins and orcomyotropin, novel partially co-localized peptides, in crayfish sensory, motor, interneuronal and neurosecretory cells

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Orcokinins (Asn^{13}-OK: NFDEIDRSGFGFN, first identified by Stangier, J., 1992, Peptides 13:859, Val^{13}-OK) and orcomyotropin (OMT: FDAFTTGFamide; Dircksen, H., et al., 2000, J Exp Biol 203:2807) isolated from crayfish CNS or hindgut extracts are hindgut-stimulatory peptides of the crayfish *Orconectes limosus*. These OKs, Ser^{9}-OK, Val^{13}-OK, Ala^{13}-OK (identified in crabs; Bungart, D. et al., 1995, Peptides 16:67), Thr^{8}-His^{13}-OK, and an OMT-like hendecapeptide occur on the same precursor in crayfish *Procambarus clarkii* (Yasuda-Kamatani, Y., Yasuda, A., 2000, Gen Comp Endocrinol 118:161). In *Orconectes*, OK-peptides and OMT are co-localized immunocytochemically to posterior median neurones of the terminal ganglion innervating the entire hindgut, to three eyestalk neurones only and to several neurones in the stomatogastric nervous system. However, OKs show much wider distributions than OMT. Hundreds of other novel OK-immunoreactive (OKir) neurones densely innervate almost all neuropils of the eyestalk, brain, suboesophageal (SOG), thoracic and abdominal ganglia, and several large projection interneurones exist. OKir cells outnumber neurones immunoreactive to other crustaceans neuropeptides. All chemosensory neurons entering the brain olfactory lobe and putative sensory neurons entering through several nerves of each ventral nerve cord ganglion and terminating in delineated neuropils strongly stain for OKir. Furthermore, mainly from a group of neurosecretory OKir neurones in anterior lateral SOG, fibres give rise to abundant neurohaemal terminals in the SOG perineurium and in the pericardial organs. This occurrence of OKir material is supported by the differential distribution of several different precursors for the OK-family peptides in different nervous tissues. Thus, putatively diverse physio-logical actions as myotropins, sensory, neuromodulatory and neurohormonal neuropeptides of different OKs alone or in a cocktail merit further exploration.

138 Ontogeny of modulatory systems

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A large body of work has been devoted to the characterization of patterns of co-localization of neuropeptides and other neurotransmitters as well as to the functional role of such co-modulation. However, little is known about their chronology of appearance within a given neuromodulatory population throughout development as well as their role in the development of neural networks. To pursue this issue, we use the stomatogastric nervous system of the lobster *Homarus gammarus* . The stomatogastric ganglion (STG) contains thirty neurons organized in networks, the expression of which

is controlled by central neuromodulatory peptidergic inputs from anterior ganglia in adult as well as in the embryo. Although the STG is made of the same neuronal population, it is organized in multiple networks and in a single motor network, respectively in the adult and embryo. Importantly, we have shown that the expression of adult and embryonic networks is different due to a differential modulatory environment. Indeed, using dye retrograde migration, we have shown that all modulatory neurons projecting to the STG in the adult system are present in the embryo as early as 65% development and already reach their adult target. Then, using retrograde dye migration coupled with immunocytochemical detection of neurotransmitters, we found it exists a progressive acquisition of neurotransmitters within this pre-established modulatory neuronal population. Furthermore, although some identified projecting neurons express their peptidergic and co-transmitters early in the development, some others acquire their transmitters in subsequent developmental stages even though they already project to their target network. These data indicate that the maturation of neural networks may rely on the ontogenetic alteration of their modulatory input neurons rather than on changes within the target networks themselves.

Multiple members of a peptide family are present in single identified neurons

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Peptides are widely distributed in the nervous system and form the largest group of neuroactive substances. One way to unravel the function of peptides is to investigate the role of identified neurons in well-studied networks. The major method used to identify neurons which contain a particular peptide is immunocytochemistry. Since most peptides belong to families which share a common amino acid sequence, antibodies generated against one member are likely to recognize other members of the same family. Therefore, using only immunocytochemistry to identify peptides present in a given neuron can be misleading. By combining immunocytochemistry with MALDI-TOF MS (matrix-assisted laser desorption time-of-flight mass spectrometry), it is possible to identify multiple members of a peptide family in single identified neurons.

We have used this combination of techniques to identify orcokinins in identified neurons of the stomatogastric nervous system (STNS). The orcokinins form a conservative family of peptides with multiple members in a given species that have extremely similar sequences. We investigated the distribution of orcokinin-like immunoreactivity in the STNS of the crayfish *Cherax destructor* and identified the orcokinins present in this species by using MALDI-TOF MS post source decay fragmentation (Skiebe et al., 2002). With MALDI-TOF MS, we detected all four identified orcokinins in the anterior median nerves, in which all the orcokinin-like immunoreactivity derives from the motoneuron AM-c6, indicating that all orcokinins are present this neuron. The masses of all four orcokinins and the peptide FDAFTTGFGHS were also detected in the inferior ventricular nerve which contains the cell bodies and axons of a pair of modulatory interneurons called the IVNs, as well as the axons of two other orcokinin-like immunoreactive neurons. Orcokinins are also likely to be present in identified sensory neurons. In

Homarus americanus, the nerve containing the axons of the PSRs (posterior stomach receptor) projects into a distinct neuropil area. Within this neuropil area, we detected the masses of all five orcokinins shown to be present in the STNS of the lobster (Li et al., 2002), as well as the mass of *Cancer borealis*-tachykinin (Christie et al., 1997). Now, we have to confirm that the PSR neurons contain these peptides. This is particularly interesting since influence of the PSR neurons on the central pattern generation in the STNS is well studied, but their transmitters are unknown. The combination of immunocytochemistry and MALDI-TOF MS allows an unambiguous identification of the peptides present in particular neurons, which is a prerequisite to study the function of peptidergic neurons in neural networks.

140 Antagonistic modulation of muscle contraction by two co-localised peptides

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We have studied the neuropeptide phenotypes of identified efferent neurons, which are present in all pereional ganglia of the marine isopod *Idotea emarginata*. Their axons project into the dorsolateral nerve, which accompanies the dorsal extensor muscles of all segments and gives rise to the pericardial organ. The neurons are allatostatin-immunoreactive in all but one pereional ganglion. However, in the ganglion of the 5th pereional segment, allatostatin staining is co-localized with FMRF-amid staining, which was confirmed with immunogold labelling. Within the pericardial organ, the allatostatin- and FMRF- immunoreactivity is associated with granules (70-100 nm), which are co-localized in numerous varicose axons providing putative release sites for the two peptides.

To investigate the consequences of the release of both peptides their modulatory effects on one of their putative targets were analysed. In experiments on the individually identifiable extensor muscle fiber 2 the crustacean FMRFamide-related peptide DF_2 enhances the efficacy of motor input to muscles through pre- and postsynaptic mechanisms. It increases EPSC amplitudes indicating that presynaptic effects contribute to an enhancement of neuronally evoked muscle contraction. Synergistically, DF_2 strongly potentiates contractions evoked by current pulses exceeding excitation-contraction threshold. In voltage-clamped fibres with Ca^{2+} replaced by Ba2+, the integrated Ba2+ current is almost doubled by the peptide, which indicates that the postsynaptic effect is supported by an increase of the high-voltage-activated inward current through L-type Ca^{2+} channels. Since DF_2 is likely to be one of the peptides labelled by the antiserum against FMRF-amide, it should result in the potentiation of muscle performance *in vivo*. Whereas DF_2 enhances muscle responses, allatostatin has the opposite effect. It decreases transmitter release from excitatory motorneurons and reduces the amplitude of evoked contractions of muscle fibers. The postsynaptic reduction of contraction under voltage clamp conditions is at least partly due to a decrease in the pharmacologically isolated inward calcium current.

We therefore consider that DF_2 and allatostatin, which can be released and distributed from identical neurons, exert converging but antagonistic modulatory actions on pre-

and postsynaptic neuromuscular parameters of an identified muscle fiber. Further studies will be aimed at investigating the possibility, that subsequent action of both peptides or selective responses of other targets may provide mechanisms for integrating and differentiating the effects of both peptides.

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Convergence and divergence of peptide cotransmitter actions: Functional consequences in a multifunctional network. 141

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We are studying the actions of peptide cotransmitters on distinct but related neural circuits in the stomatogastric nervous system of the crab. The modulatory projection neuron MCN1 uses GABA and the peptides proctolin and CabTRP Ia to elicit/modulate the gastric mill and pyloric rhythms in the stomatogastric ganglion (Blitz et al, J Neurosci 1999). The actions mediated by both peptides converge on nearly all pyloric neurons (Nusbaum et al, TINS 2001) and both elicit similar currents (Swensen & Marder, J Neurosci 2001). We are now studying if convergent cotransmitter actions are also prominent in the MCN1 actions on the gastric mill circuit.

Both, focal transmitter application and MCN1 stimulations show divergent cotransmitter actions on the gastric mill circuit. CabTRP Ia, proctolin and GABA influence overlapping and distinct sets of gastric mill follower neurons, while MCN1 excites the core pattern generator neurons [the reciprocally inhibitory neurons INT1 and LG] via only GABA (excites INT1) and CabTRP Ia (excites LG). MCN1 does not have a direct proctolinergic action on the gastric mill core pattern generator. However, MCN1 probably does use proctolin to modulate the gastric mill rhythm, albeit indirectly. MCN1 excites the pyloric rhythm via the actions of proctolin, and the pyloric rhythm influences the gastric mill rhythm cycle frequency (Bartos et al, J Neurosci 1999). We are currently testing the possibility of this indirect proctolin action. Thus, a modulatory projection neuron uses convergent cotransmitter actions on one circuit and divergent cotransmitter actions to influence a separate but related circuit, while at the same time both circuits influence each other.

Dissecting and modeling the actions of neuromodulatory peptides on multiple targets in a network of identified neurons 142

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Neuromodulatory peptides often affect multiple cellular and synaptic targets in the same network. We used the dynamic clamp and computational models to understand how such modulator-induced changes in cell and synapse properties contribute to the changes in network output caused by peptide application.

The peptide Red Pigment-Concentrating Hormone (RPCH) is present in the stomatogastric ganglion (STG) of the lobster *Homarus americanus* both as a hormone and due to release from peptidergic interneurons. We demonstrate that RPCH greatly increases the strength of the only feedback synapse to the pacemaker kernel of the pyloric pattern generating network. At the same time, RPCH also increases the duration of the inhibitory synaptic feedback signal by increasing the burst duration of the presynaptic neuron, the lateral pyloric (LP) neuron. Finally, we show that the pacemaker kernel itself is also a target of modulation by RPCH. How do these three neuromodulatory effects of the same peptide act together to slow the rhythmic output pattern of the pyloric network?

In biological networks it is often difficult to study the different actions of a modulatory substance in separation. We used the dynamic clamp to overcome this problem by replacing the synaptic feedback synapse onto the pyloric pacemaker with an artificial synaptic input whose strength and duration can be varied independently. These dynamic clamp experiments showed that a dramatic increase in synapse strength – like the one caused by RPCH in the feedback synapse from LP – does not necessarily change the functional impact of the feedback signal on the postsynaptic pacemaker neuron. In contrast, moderate changes in the duration of synaptic input to a pacemaker or neuromodulation of the pacemaker itself can more reliably affect the period of the rhythmic network output.

These results are confirmed by computational studies on a bursting Hodgkin-Huxley type model neuron. Again, dramatic increases in the strength of inhibitory synaptic input to the model pacemaker neuron do not necessarily affect the pacemaker period while increases in the duration of the synaptic input always lead to increases in the burst period. Furthermore, the computational model allowed us to identify specific properties of underlying membrane currents as the cause of this relative insensitivity of pacemaker neurons to increases in synaptic input strength. In addition, we were able to generalize this finding to excitatory inputs and to tonically spiking neurons, indicating that a wide catgory of periodically firing neurons is more sensitive to the temporal aspects than to the amplitude of synaptic input.

In the case of modulation of the pyloric circuit by RPCH our results demonstrate that while all three effects of the peptide contribute to the slowing of the network output, it is not the very dramatic increase in feedback synapse strength caused by RPCH, but rather the fairly modest increase in feedback duration that modulates the network output most reliably.

Introductory Remarks to Symposium 16

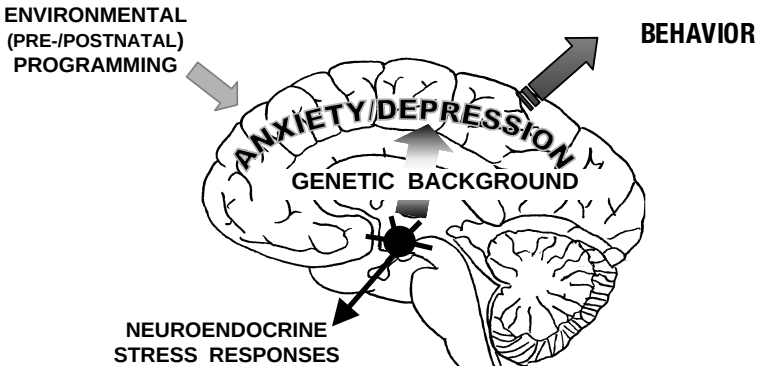
Early environmental programming: Molecular, neuroanatomical, neuroendocrine and behavioural effects

Inga Neumann and Katharina Braun

There is profound evidence that the development and manifestation of various psychiatric diseases, such as major depression or anxiety disorders as well as cognitive deficits are dependent on prenatal and immediate postnatal factors. Whereas the basic wiring of the mammalian central nervous system is genetically programmed, its fine tuning throughout different phases of infancy, childhood, and adulthood are highly dependent on environmental conditions. Early experiences which occur during phases of elevated neuronal and synaptic plasticity appear to „imprint"" patterns of synaptic connectivity, neural circuitries, and neuronal and neuroendocrine activity in the infant brain. In particular the wiring patterns of limbic circuits, which are relevant for learning and memory, emotional behavior and regulation of neuroendocrine stress responsiveness are modified by early emotional experiences.

The symposium will present evidence that exposure to pre- or postnatal stress permanently modifies brain functions at various levels. In the prenatal period, pharmacological or physiological exposure to excessive levels of glucocorticoids, which can easily cross the placentar-barrier, is known not only to reduce birth weight but also to be associated with an increased risk of cardiovascular, metabolic, neuroendocrine and emotional pathophysiologies in adulthood. Jonathan Seckl will demonstrate the importance of the placentar glucocorticoid „buffer"" enzyme 11β-hydroxysteroid dehydrogenase in preventing the effects of prenatal stress relevant for rodents and humans. With respect to stressful emotional experiences during the early postnatal period, Katharina Braun will present data from two rodent models which indicate that repeated or chronic parental separation affects the synaptic reorganization in the anterior cingulate cortex, hippocampus and amygdala.

New insights into the influence of early postnatal maternal deprivation versus handling on the development of neuroendocrince function including the behavioural corre-



143 Prenatal glucocorticoid programming of adult brain and body

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Epidemiological studies in distinct populations associate low weight or thinness at birth with hypertension, insulin resistance/type 2 diabetes and ischaemic heart disease in adult life. The concept of fetal 'programming' has been advanced in explanation, but the underlying mechanism(s) are unclear. Fetal overexposure to glucocorticoids (GCs) may, in part, explain the observations. In rats, birth weight is reduced following prenatal exposure to the synthetic GC dexamethasone, which readily crosses the placenta, or to carbenoxolone, which inhibits 11ß-hydroxysteroid dehydrogenase type 2 (11ß-HSD2), the physiological feto-placental 'barrier' to the higher levels of GCs in the maternal circulation. Whilst the offspring regain the weight deficit by weaning, as adults they exhibit permanent hypertension, hyperglycaemia and increased hypothalamic-pituitary-adrenal (HPA) axis activity. Physiological variations in placental 11ß-HSD2 activity near term correlate directly with fetal weight. The critical 'window' for GC programming appears to be the last third of gestation. In humans, 11ß-HSD2 gene mutations cause low birth weight and several studies show reduced placental 11ß-HSD2 activity in association with intrauterine growth retardation. Moreover, low birth weight babies have higher basal and ACTH-stimulated plasma cortisol levels throughout adult life indicating HPA axis programming. The molecular mechanisms may reflect permanent changes in the expression of specific transcription factors, key amongst which is the glucocorticoid receptor (GR) itself. The differential programming of GR in different tissues reflects effects upon one or more of the multiple tissue-specific alternate first exons/promoters of the GR gene. Overall, the data suggest that both pharmacological and physiological exposure to excess GCs in the prenatal period can programme cardiovascular, metabolic and neuroendocrine pathologies in adult life.

144 Effects of parental separation on the maturation of limbic circuits

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In sensory and motor cortices the synaptic development of cortical function has been shown to be modulated by early environmental influences. We postulate that, in analogy, early emotional experience may modify and shape synaptic connectivities within cortical and subcortical limbic circuits. Thus, the aim of this study was to assess the effect of different degrees of sensory and socio-emotional complexity on the functional maturation of synaptic circuits in the limbic system of the trumpet-tailed rat Octodon degus.

In 21 day old pups the densities of dendritic spines were quantified in pyramidal cells in the anterior cingulate cortex (ACd) the hippocampal area CA1 and in the lateral nucleus of the amygdala, as well as in granular cells of the dentate gyrus. Spine densities were compared between normal control animals, who lived undisturbed with their families, and deprived animals, which were individually separated from the parents once per day for one hour between day 1 to 21.

Compared to the control animals the deprived animals showed increased spine densities in the ACd and in the CA1 region (5.5%). In contrast, spine density in the dentate gyrus was reduced (8.5%) in the deprived group, no changes were found in the lateral amygdala. In contrast, spine development in the somato-sensory cortex, which served as control region in this study, was not affected by parental separation.

These results reveal region-specific modulations of synaptic inputs in limbic areas, which are most pronounced in the limbic cortical structures, thus confirming our assumption that early emotional experience shape limbic synaptic networks. There is evidence that such experience-induced synaptic changes last until adulthood, suggesting that they can determine and/or limit cognitive and psychosocial capacities.

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Adaptive neuroplasticity to perinatal stressors: Morphology, neurobiology and behavior. 145

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Functional development of the central nervous system is the result of a continuous interaction between genetic and environmental influences. Accumulating evidence points to perinatal and childhood adversity, acting in conjunction with genetic liabilities, as significant risk factors for development of mood disorders and medical diseases. In an ongoing series of studies, we have compared the neurodevelopmental trajectories in rodent and nonhuman primate models including prenatal social instability, neonatal maternal separation, and naturally occurring maltreatment in order to elucidate ?sensitive periods? in development, characterize neurocircuits vulnerable to maladaptation, and to identify genes potentially associated with individual vulnerability (or resilience) to changes in the quality of experience-expectant input associated with care-giving. The neurocircuits underlying each of these processes may be viewed as interrelated targets whose setpoints can be programmed by external stimuli, giving rise to systems that may respond differently to the same stimulus with respect to developmental stage. Many of the long-term effects of adverse early experience are intriguingly similar in rodents and nonhuman primates including increased anxiety-like behavior, anhedonia, as well as deficits in social behavior, sexual behavior, and cognitive performance. Specific adaptations occur in neurocircuits at the level of structure, gene, and protein expression. The prefrontal cortex, hippocampus, bed nucleus of the stria terminalis, amygdaloid complex, nucleus accumbens, locus coeruleus, and raphe nuclei appear particularly plastic during development and early childhood. These regions exhibit changes in neuromorphology, neu-

rotransmitter expression and content, receptor mRNA and binding, and in intracellular signaling cascades. Neonatal maternal separation is associated with an approximately 50% decrease in hippocampal DG neurogenesis when evaluated at postnatal day 21. Dendritic complexity, as determined by analysis of branching, is also significantly decreased in hippocampal subregions. The long-term trace of this reduced complexity during development appears to be a reduction in synaptic density as determined by electron microscopy and expression of synaptogranin mRNA in adults. The most affected neurotransmitter systems include the corticotropin releasing factor (CRF), γ aminobutyric acid (GABA), and noradrenergic systems, which are affected in a region specific manner. Expression profiling has revealed gene expression changes in elements of DNA-RNA synthesis (DNA methyltransferase), neuronal growth and adhesion (cahedrin, N-CAM, BDNF, NGFI-A), intracellular signaling cascades (PKC α, MAP kinase kinase), glial markers (GFAP), and numerous neuronal signaling systems. These gene changes suggest alterations in plasticity and intracellular signaling cascades. This cascade of adaptations leads to changes in perception and processing of the salience of external stimuli and, thus, the response to them as well as sensitization of stress-responsive neurocircuits. This strategy should prove useful in identifying potential ?windows of opportunity? for targeted interventions and common targets for pharmaceutical treatments. (Supported by NIH grants MH50113, MH62577, MH065046 and The Silvio O. Conte Center for the Neuroscience of Mental Disease grant MH58922).

146 Molecular and neuroendocrine effects of maternal deprivation in mice lacking the CRH receptor type 1

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During postnatal development, mice undergo a so-called stress hyporesponsive period (SHRP), which is characterised by low basal corticosterone levels and the inability of mild stressors to induce a corticosterone response. It has been shown, that the quiescence of the HPA axis during the SHRP is regulated by maternal factors and that a disruption of the mother-infant interaction can activate the HPA system. A separation of the mother from the pups for 24 hours (maternal deprivation) has been demonstrated to result in a variety of short and long term consequences. However, the molecular mechanism of the observed changes is poorly understood. It has been hypothesised, that the CRH receptor 1 (CRHr1) may play an important regulatory role during development by mediating the effects of maternal deprivation. We therefore applied the maternal deprivation paradigm to different CRHr1 deficient mouse lines, in order to examine the role of this receptor on the effects of maternal deprivation and in regulating the expression of HPA axis related genes. We could demonstrate, that the CRHr1 is essential for the activation of the corticosterone response following maternal deprivation, most likely via CRHr1 in the pituitary. Furthermore we could show, that the CRHr1 is directly involved in the regulation of CRH expression in the PVN and MR expression in the hippocampus. In contrast, effects of maternal deprivation on GR expression are not mediated by this

receptor. In conclusion, our data enable us to create a hypothesis about the direct molecular and neuroendocrine effects of maternal deprivation.

Effects of early life stress: Dependency on gender and the genetic predisposition to high and low anxiety

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Repeated exposure to stress in utero (prenatal stress; PS) or to periodic maternal deprivation (PMD) in the immediate post partum period has been shown to chronically alter emotional and neuroendocrine measures of the offspring. Here we tested the hypothesis that genetic factors may interact with such early life experiences comparing male and female Wistar rats (Charles River, Sulzfeld, Germany; study 1) and comparing Wistar rats selectively bred for high (HAB) and low (LAB) anxiety-related behaviour (study 2).

For PS, pregnant dams were daily exposed to psychosocial (maternal defeat as a relevant emotional stressor for female rats, 15 min) and emotional (restraint, 45 min) stressors between days 5 and 20 of pregnancy. For PMD, litters were daily separated from their mother for 3 hours. At the age of 12 weeks, the anxiety-related behaviour on the elevated plus-maze (EPM) and/or on the modified holeboard, as well as the responsiveness of the hypothalamo-pituitary-adrenal (HPA) axis to a mild stressor were quantified.

In study 1, PMD significantly increased the level of anxiety in adult male (70% reduction in the percentage of entries into the open arms of the EPM) and female (31% reduction) rats in a gender-dependent manner. Further, the HPA axis response to an emotional stressor was significantly elevated in male ($p<0.01$), but not female PMD-treated rats compared to their respective untreated controls. These results indicate that the vulnerability of male rats to postnatal stress triggering emotional and neuroendocrine alterations is higher compared to female rats.

In study 2, exposure of male HAB and LAB rats to PS or PMD resulted in contrasting effects on anxiety-related behaviour in adulthood with HABs becoming less and LAB rats becoming more anxious compared to their unstressed controls. Thus, early life stress attenuated the extreme behavioural traits in both lines. Further, after PMD, the hyperresponsiveness of the HPA axis to acute stressors accompanying hyper-anxiety became attenuated in HAB rats, whereas, in LAB rats, HPA axis responses tended to increase after PMD. The evolutionary advantage of these opposing effects of early life stress may be that the genetic variability among individuals of a species is sustained while allowing flexible and adequate responses to stressful and potentially dangerous stimuli in adulthood.

Thus, we can provide strong experimental evidence for differential effects of exposure to early life stress on adult behavioral and neuroendocrine stress responses which are dependent upon the genetic predisposition of the animal.

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149 Setting apart the affected: The use of behavioral criteria in animal models of Acute Stress Response and Post Traumatic Stress Disorder

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Post-traumatic stress disorder affects only 20-30% of the exposed population. Clinical studies of acute and chronic responses to stress generally employ stringent criteria for inclusion in the study population, and yet in animal studies the data are usually expressed as a function of the entire exposed versus the entire unexposed populations, regardless of individual variation in response. Prior data support an approach to animal models analogous to inclusion criteria in clinical studies.

This series of studies sought to assess prevalence rates of maladaptive versus adaptive responses determined according to two successive behavioral tests (elevated plus maze/open field and acoustic startle response tests), amongst rats exposed to a variety stress paradigms, in the acute and chronic phases, for single and repeated exposures, early in life and/or in adulthood.

The results shed light on the pattern of prevalence rates of maladaptive responses to stress over time, with rates dropping from 90% acute responders to a plateau at about 25% enduring/chronic response. This plateau is attained at seven days and remains unchanged at 30 days in two distinct study models. Different types of trauma are associated with different degrees of response, possibly reflecting a "dose-response" relationship to severity of trauma. Re-traumatization is associated with increasing prevalence of maladaptive responses, all the more so for early-life trauma repeated in adulthood.

Setting the affected individuals apart from the unaffected and focusing on them clarifies the overall picture and more closely approximates clinical studies.

150 Quantitative analysis and 3D-reconstruction of neuronal and synaptic structures from serial sections

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Our previous light confocal lasercsan- and electron microscopic studies in intact animals and cell cultures (Helmeke C, Ovtscharoff jr et al., 2001, Cerebral Cortex, 11: 717-727; Braun K, Segal M, 2000, Cerebral Cortex, 10:1045-1052) have demonstrated that early stressful experience, such as parental separation can induce significant changes in synaptic numbers and composition in limbic cortical and subcortical areas. The aim of

this work was to further analyze changes of synaptic size and shape in more detail, using three dimensional reconstruction of cultivated hippocampal neurons.

Up to date is not much known about synaptic formation. Single neurons were stained with Uranyl Acetate and serial sections (120nm) were imaged at high magnification using a high resolution CCD camera attached to the electron microscope LEO 912. After alignment of sectioned series using SEM software, the neurons and their fine details (dendrites and spines) were reconstructed using IGL software, and volume and surface were calculated to present a 3D objects based on sTEM. Displayed realistic objects of the fine ultrastructure can be combined using a various orientations, surface rendering and rotation. This 3D objects can be viewed using Web browsers capable of interpreting VRLM, such as Netscape with Silicon Graphics Cosmo Player plug- in. rams.

Supported by DFG

Consequences of maternal separation during different stages of early development on HPA axis activity in three week old rats. 151

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The hypothalamo-pituitary-adrenal (HPA) axis plays a central role in the regulation of the stress response. In the neonatal period, the ability of rat pups to respond to stressful stimuli is immature, and includes a stress-hyporesponsive phase (SHRP). The specific developmental pattern of HPA axis can be disrupted by different stimuli including maternal separation, an complex psychological and physiological stressor. The aim of the present experiment was to study the consequences of maternal separation during different developmental stages, i.e. prior, during, and after the occurrence of the SHRP, on basal and novelty-induced HPA axis activity in juvenile, three week old rats.

At the day of birth (postnatal day (PND) 0) litters were culled to 12 pups/ mother and at PND1 litters were randomly allocated to one of the 4 experimental groups: (i.) controls (reared undisturbed); (ii.) iso 1-3: pups were individually separated for 1 h/ day at PND 1-3 (prior to SHRP); (iii.) iso 5-7: same treatment as (ii.) but at PND 5-7 (during SHRP); or (iv.) iso 14-16: same treatment as (ii.) but at PND 14-16 (after SHRP). At PND 21 male pups of all experimental groups were allocated to one of the following stress treatment paradigms: a.) basal: animals were decapitated within 10 sec after removal from the home cage and trunk blood was collected; samples were assayed for ACTH and corticosterone using commercially available kits; b.) novelty: animals were placed in an open field for 30 min; then the animals were decapitated and trunk blood was collected; c.) novelty and back to family: after 30 min of open field experience, the animals were given back to family (mother and remaining pups) for a period of 45 min after which trunk blood was collected.

The comparison of the basal levels between the four experimental groups revealed no significant differences for ACTH, but enhanced levels of corticosterone in the iso 1-3 ($p<0.05$) as well as iso 14-16 ($p<0.05$) group compared with controls or the iso 5-7

group. Furthermore, the novelty experience resulted in significantly enhanced levels of ACTH and corticosterone compared with those during basal ($p<0.05$) and after novelty and back to the family condition ($p<0.05$). Taken together, these findings reveal the specific impact of maternal separation carried out during different stages of ontogeny on basal HPA axis activity. Whereas no difference in the reactivity of HPA axis towards novelty was measurable in all groups, these results indicate the detrimental effect of maternal separation before and after SHRP on basal corticosterone levels of three week old rats. Further studies are needed to examine the effect of those enhanced glucocorticoid levels generated early in life for further development. Supported by Volkswagenstiftung.

152 Early traumatic experience alters metabolic brain activity in thalamic, hypothalamic and prefrontal cortical brain areas of *Octodon degus*

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Juvenile, emotionally modulated learning events, such as the formation of an emotional bond between a newborn animal and its mother, are fundamental for the establishment and maintenance of synaptic networks in the developing brain. Early deprivation and traumatic experiences can interfere with these events, resulting in under- or maldeveloped brain circuits, which cause enduring behavioral and cognitive impairment. Based on a previous study we applied the 2-fluorodesoxyglucose (2FDG)-technique to look for changes in metabolic activity of thalamic, hypothalamic and prefrontal cortical brain areas during juvenile traumatic experiences in 8 day old Octodon degus pups. Metabolic activation, densitometrically measured as 2-FDG uptake, was compared between four experimental groups: i) pubs, that were left undisturbed with their parents in the home cage, ii) pups, that were separated as a group of littermates iii) pups, that were individually separated for 45 minutes from the parents, iv) pups, treated as group iii but during separation stimulated with maternal vocalizations. We found significant alterations of metabolic brain activity in all separated groups in thalamic and hypothalamic brain areas and in the orbitofrontal cortex. In principal there was a strong reduction of brain activity in the separated animals compared to the degu pups reared with their parents. However, there were also differences between the animals of the three separated groups. The individually isolated animals (group i) showed a more pronounced reduction of 2-FDG uptake than the degu pups separated together with their littermates (group ii) and the degu pups stimulated with maternal vocalizations during separation (group IV). Since neuronal activation regulates the development of synapses during postnatal brain maturation, the disturbed activation during stressful situations may interfere with the refinement of limbic connectivity patterns, resulting in altered processing of emotional stimuli in adulthood.

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Introductory Remarks to Symposium 17

New forms of cerebellar signaling

Jana Hartmann and Arthur Konnerth

Recent years have revealed unexpected mechanisms of neuronal signaling in the cerebellum. Particularly retrograde signaling at Purkinje cell synapses has triggered a wide interest among neuroscientists. Depolarization-induced suppression of inhibition (DSI) is a calcium-dependent transient suppression of transmitter release following postsynaptic depolarization at the interneuron-Purkinje cell synapse. Previous work of Alain Marty and colleagues indicated that DSI is induced by the release of a retrograde messenger. Alain Marty will present new data, which allow a quantitative estimate of the changes in interneuron excitability and the probability of release at interneuron-Purkinje cell synapses connected with DSI. Wade Regehr and coworkers have demonstrated that DSI is not restricted to inhibitory synapses but presents a more general type of synaptic regulation. They identified endogenous cannabinoids as the retrograde messenger responsible for DSI and depolarization-induced suppression of excitation (DSE) in the hippocampus and cerebellum, respectively. In his talk Wade Regehr will focus on new results from his laboratory about the role of the endocannabinoid system at Purkinje cell synapses. At excitatory Purkinje cell synapses either calcium influx following postsynaptic depolarization or calcium release from intracellular stores due to activation of postsynaptic metabotropic glutamate receptors (mGluRs) is sufficient to induce the production of endocannabinoids. This putative synergistic action of both pathways in the regulation of transmitter release is suggested by recent findings from Masanobu Kano's laboratory and will be the topic of his presentation. The role of presynaptic calcium stores in synaptic transmission at inhibitory interneuron-Purkinje cell synapses will be discussed by Isabel Llano. She and her colleagues detected for the first time spontaneous ryanodine receptor-mediated calcium transients in presynaptic terminals, a mechanism that will be covered in her presentation. Jana Hartmann will review recent findings about neurotrophin-mediated signaling in Purkinje cells. Brain-derived neurotrophic factor (BDNF) has a rapid excitatory action in Purkinje cells and is capable of LTD-induction. Thus, in the cerebellum BDNF is a transmitter-like signaling molecule that modifies persistently synaptic transmission even in response to a single brief exposure. The cerebellar cortical output is shaped by the interplay between Purkinje cell somata and dendrites. Mechanisms for coincidence detection between parallel fiber and climbing fiber inputs in dendrites of Purkinje cells will be described by Michael Häusser. Based on results from *in vivo* dendritic patch-clamp recordings he will show the relation between patterns of synaptic activity and specific patterns of spiking.

153 Mechanisms of retrograde synaptic modulation at interneurone-Purkinje cell synapses

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Depolarization-induced calcium elevations in Purkinje cells lead to a sequence of presynaptic inhibition, called depolarization-induced suppression of inhibition (DSI), and of postsynaptic potentiation, called rebound potentiation (RP). DSI was studied quantitatively using synaptically connected pairs where presynaptic interneurones were submitted to perforated patch recording while postsynaptic Purkinje cells were studied with conventional whole-cell recording. During DSI, the weight of action potential independent IPSCs increased from 25% in control conditions to about 50%. DSI involved an increase in the failure rate (from 1 to 20%), in the paired pulse ratio (from 96 to 133%) and in the variance to mean ratio (by a factor of 2.17-fold). If the presynaptic cell was dialysed with Cs+, the mean amplitude of the evoked IPSC increased 2.71-fold, and the variance to mean ratio decreased 2.6-fold. DSI was much reduced (29.2%), and could only partially be recovered (to 46.6%, while the variance to mean ratio recovered entirely) by decreasing the extracellular Ca^{2+} concentration so as to match the amplitude of the evoked current. These results combined with those of Kreitzer et al. (2002) indicate that several presynaptic processes contribute to DSI: reduction in the frequency of miniatures (by 13% of the total), of the frequency of the presynaptic action potentials (by 14%), and of the probability that presynaptic depolarizations elicit transmitter release (by 73%). The latter component involves a modulation of K^+ channels and trial-to-trial modifications of the presynaptic signal.

154 Retrograde modulation of synapses by endocannabinoids

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Purkinje cells, which are the sole output of the cerebellar cortex, receive inhibitory synapses from interneurons in the molecular layer and excitatory inputs from climbing fibers that originate in the inferior olive and granule cell parallel fibers. Elevations of calcium in Purkinje cell dendrites result in the formation and liberation of endocannabinoids in a process that does not involve vesicle fusion. These bind to presynaptic cannabinoid receptors to suppress all excitatory and inhibitory synapses onto Purkinje cells for tens of seconds.

Progress has been made in identifying the mechanisms of endocannabinoid modulation. Optical measurement of presynaptic calcium entry revealed that suppression of excitatory transmission arises from presynaptic calcium channel inhibition. In contrast, multiple mechanisms contribute to the suppression of inhibitory synaptic transmission. In addition to directly modulating nerve terminal calcium influx, endocannabinoids released from Purkinje cell dendrites suppress inhibitory synapses is by reducing the firing rate of nearby interneurons. This is a result of cannabinoid-receptor-dependent activation of a

potassium conductance in interneurons. Because interneurons project several hundred microns, this reduction of firing allows cells to regulate synaptic inhibition at distances well beyond limits set by diffusion.

The dependence of endocannabinoid release on postsynaptic calcium levels, the synapse specificity of modulation, the physiological role of retrograde signaling by endocannabinoids and the widespread role of endocannabinoid modulation will be discussed.

Endocannabinoid-mediated retrograde signaling triggered by activation of postsynaptic metabotropic glutamate receptors in cerebellar Purkinje cells 155

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Cannabinoid CB1 receptors (CB1Rs) are thought to mediate multiple effects of Marijuana. Recent studies have clarified that endogenous cannabinoids are released from the postsynaptic neuron and act retrogradely onto the presynaptic CB1R to regulate transmitter release. In cerebellar Purkinje cells (PCs), we found that activation of metabotropic glutamate receptor subtype 1 (mGluR1) caused a transient suppression of transmitter release from excitatory synaptic terminals. This effect was abolished by inactivating G proteins in postsynaptic PCs and deficient in *mGluR1* knockout mice. The deficiency was restored by introducing *mGluR1* into PCs of the knockout mice. This suppression was occluded by a cannabinoid agonist (WIN55,212-2) and was totally abolished by cannabinoid antagonists (AM281 and SR141716). In addition, depolarization-induced Ca^{2+} transients in PCs causes transient suppression of transmitter release at excitatory synapses, which is also mediated by the presynaptic CB1R. These results strongly suggest that endocannabinoids are produced in PCs by either mGluR1 activation or Ca^{2+} elevation, act retrogradely onto the presynaptic CB1R and reduce transmitter release. These two pathways may function in a synergistic manner *in vivo* to regulate transmitter release depending on the activity of postsynaptic PCs.

Probing the role of intracellular calcium stores in presynaptic calcium signalling 156

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Two-photon calcium imaging of GABA-releasing interneurones in cerebellar slices was used to investigate the contribution of intracellular calcium stores to presynaptic calcium signalling. In order to identify potential pre-synaptic terminals, we measured the calcium-dependent fluorescence changes elicited by short trains of action potentials, in

the axonal arbour of interneurones dialysed with calcium-sensitive probes through patch-clamp pipettes. As previously shown, action potential evoked axonal calcium rises have a highly heterogeneous spatial profile, characterised by "hot spots" along the axonal arbour and in specialized regions corresponding to synaptic terminals formed onto the somata of Purkinje cells. In the presence of tetrodotoxin, these axonal sites present spontaneous calcium transients (SCaTs) which have a random occurrence and a highly localised spatial profile. The addition of ryanodine (1 to 10 μmolar) to the bath solution leads to an increase in the frequency of occurrence of SCaTs, thus arguing for ryanodine-sensitive intracellular calcium stores as their source of calcium. The co-localisation of SCaTs and action potential evoked calcium rises indicates that functional ryanodine receptors are constrained to putative presynaptic terminals. Analysis of the spatial and temporal profile of SCaTs under different experimental conditions will be discussed in terms of the implications of this novel type of calcium signalling for synaptic transmission.

157 BDNF-mediated rapid signaling in cerebellar Purkinje cells

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Brain derived neurotrophic factor (BDNF) is a member of the neurotrophin-family, which regulates neuronal survival and differentiation, and also modulates activity-dependent neuronal plasticity. BDNF was shown to have a rapid excitatory action that was particularly strong in rat hippocampal and cortical neurons (Kafitz et al., 1999). The underlying depolarization was found to result from a TrkB-mediated activation of the sodium channel $Na_V1.9$ (Blum et al., 2002).

Here we report results obtained in cerebellar slices of adult mice. Whole-cell patch-clamp recordings were combined with confocal calcium imaging and BDNF was pressure-ejected locally to different subcellular compartments of Purkinje cells. As in hippocampal and cortical neurons, BDNF was found to evoke a rapid inward current. The current was particularly large in the terminal spiny branchlets of the dendritic tree. The BDNF-evoked electrical response was associated with a transient rise of the cytosolic calcium concentration. Short BDNF-application pulses (50 - 200 ms) evoked large calcium transients – reaching amplitudes of tens of μmolar – in spiny dendrites of cerebellar Purkinje cells. The calcium transients were confined to local dendritic domains at the site of BDNF-application. Both the electrical response and the calcium signals were blocked by saxitoxin, but not by tetrodotoxin. These results demonstrate for the first time the presence of sodium channels in the remote dendrites of cerebellar Purkinje cells. Interestingly, while the cell body and the primary dendrites were found to express voltage-gated sodium channels, our findings suggest that the higher order dendrites express ligand (=BDNF)-gated sodium channels. Our results further indicate that the BDNF-evoked calcium signals are generated by the activation of voltage-gated calcium channels.

It was previously shown that BDNF mediates long-term potentiation (LTP) in the hippocampus and cortex. Surprisingly, at the excitatory parallel fiber/Purkinje cell synap-

ses, a single BDNF-pulse (< 1s) evoked long-term synaptic depression (LTD). Induction of LTD did not require coincident activity of parallel fibers.

Taken together, our results reveal several new actions of the neurotrophin BDNF in adult mice. Our results strongly suggest that in the cerebellum BDNF is a transmitter-like signaling molecule, with a key role in the regulation of synaptic transmission.

Dendritic integration in cerebellar Purkinje cells 158

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Cerebellar Purkinje cells receive excitatory input from 200,000 parallel fibres (PF) and a single climbing fibre (CF). The way in which Purkinje cells integrate these inputs to generate the output of the cerebellar cortex is not well understood. We have made simultaneous somatic and dendritic patch-clamp recordings from Purkinje cells in cerebellar slices in order to determine how the properties of the dendritic tree influence integration. At subthreshold potentials, PF synaptic inputs were amplified by voltage-gated sodium channels located near the soma. At depolarized potentials above spike threshold, however, both PF and CF inputs could trigger calcium spikes and plateaus which originated in the dendrites. Coincident activation of PF and CF inputs triggered dendritic spikes which were initially localized to near the site of synaptic input, but which could spread widely, depending on the level of depolarization or the pattern of PF input. These non-linear interactions could shape the pattern of axonal output generated by both PF and CF inputs, indicating that regulating the interplay between Purkinje cell somata and dendrites can help shape cerebellar cortical output. These results were complemented by patch-clamp recordings from Purkinje cell dendrites *in vivo*, demonstrating how physiological patterns of synaptic activity can gate specific patterns of spiking.

Role of thyroid hormone in Purkinje cell dendritic development 159

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Thyroid hormones (TH) exert profound effects during cerebellar development. Perinatal TH-deficiency leads to severe developmental pertubations including a reduction of growth and branching of Purkinje cell dendritic arborization, delayed granule cell proliferation and migration, shorter granule cell paralell fibers, delayed myelination, and changes in synaptic connections among cerebellar neurons and afferent neuronal fibers. In rodents, all of these abnormalities are completely reversible if TH is replaced within the first two postnatal weeks. The molecular mechanisms underlying these TH effects are poorly understood.

The cerebellar cell culture system provides an excellent model by which to study the effects of TH, since the development of cerebellar cells in mixed as well as as purified

cultures is well characterized. Addition of different concentrations of TH to mixed cultures revealed a dramatic increase in Purkinje cell dendrite branching and caliber in a dose- and time-dependent manner. This effect seems to be mediated by thyroid hormone receptor (TR)α1 since Purkinje cells in mixed cultures derived from TRalpha1 $^{-/-}$ mice do not respond to TH treatment, while Purkinje cells from TRbeta $^{-/-}$ mice show the same TH responsiveness as control cells. Furthermore, TH- induced dendritic growth is even diminished if purified Purkinje cells from TRalpha1 $^{-/-}$ mice are cocultured with wildtype granule cells and/or glia cells. These results suggest that TH directly induces dendrite outgrowth of Purkinje cells by acting via TRalpha1.

160 Mixed excitatory/inhibitory effect of $GABA_A$ synapses in the cerebellum

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In the molecular layer of the cerebellum of rats (at 12-15 and at 20-25 days), there are basket and stellate cells, GABAergic interneurons which make synapses upon the Purkinje cells and between themselves. Through gramicidin perforated patches, we could measure the mean reversal potential of $GABA_A$ conductances (E_{GABA}) for Purkinje cells and for interneurons, which respectively equal – 85 mV and – 58 mV. So, the $GABA_A$ synapse upon the Purkinje cell is clearly inhibitory.

For the interneurons, the measured mean cell membrane potential V_m varies between –75 and –50 mV among interneurons. There is so an overlap between values of V_m and E_{GABA} indicating that the $GABA_A$ synapse upon interneurons can be excitatory or inhibitory.

Indeed, using the fact that interneurons fire spontaneously, we show that extracellular stimulation of $GABA_A$ presynaptic fibers induce both excitatory and inhibitory effect upon the measured postsynaptic interneuron in all of the following recording conditions : whole-cell current clamp configuration, gramicidin current clamp perforated patch configuration or cell-attached configuration.

These mixed connections between interneurons can play as a frequency buffer among interneurons of the molecular layer, or can allow different types of time correlation between Purkinje cells, the axons of which are the only output of the cerebellar cortex.

Introductory Remarks to Symposium 18

Complex sensory processing in the vertebrate midbrain

Bernhard Gaese and Harald Luksch

Analyzing sensory information and creating useful behavioral output from it is, in short, the general function of the brain. Although all parts of the brain are involved in this task, the production of complex behavior in avian and mammalian vertebrates is usually thought of as being strongly related to forebrain structures. The midbrain, on the other hand, is mainly considered to be involved in the production of simple orienting behavior, e.g. saccadic eye movements. The aim of this symposium is to broaden the scope by taking together recent results showing how midbrain structures are involved in rather complex processing, how these structures integrate information from different sensory systems, and how the processing is connected to that in other sensorimotor loops.

We will start with an overview of the key elements for visual processing in the pigeon optic tectum. Based on a detailed analysis of the anatomical connections this will show how the retinotopically mapped input to the tectum is transformed into topographies more closely related to specific functions such as movement and looming detection (Güntürkün). It will then be shown how the processing of one important aspect of visual stimuli, visual motion, is carried out at the cellular level in the optic tectum of birds, and how the biophysical features of neurons and local networks are optimized for such a specific processing (Luksch). The mammalian counterpart of the avian optic tectum, the superior colliculus, has been analysed in comparable detail. Examples of neuronal circuits and related molecular structures involved in visual processing will be presented, with an emphasis on the inhibitory activity and its importance for the processing within and in between midbrain nuclei (Schmidt). The visual input into these structures is, among others, used to generate orienting responses towards objects of interest. Usually large sets of distributed neurons are involved in this visuo-motor transformation. Principles of processing underlying the integration of such distributed activity was analyzed in the cat and rat (Engler, Kang, Brecht, and Engel). In addition to the predominant visual afferents, the superior colliculus is a centre for the integration of input from different sensory modalities. As an example, the processing of auditory spatial activity and its organization into a map of auditory space in the mammalian superior colliculus making use of the virtual space technique will then be presented (King). Finally, the integration of orienting behavior into the actual behavioral context is discussed. This is done by presenting new data on the visual-auditory integration subserving the spatial orienting of attention in barn owls and rats. The attention-related activity, found already at the midbrain level, is seen as one of several mechanisms ensuring a consistent behavioral output by integrating sensory input from different modalities and from different levels of analysis (Gaese).

Taken together, these presentations demonstrate how complex the processing of information at the midbrain level is, and how the resulting activity is integrated into the entire network of the brain to produce adaptive behavior.

161 Retinotopy to Functionotopy: Structural Organization of Parallel Information Processing Within the Tectofugal Visual System of Pigeons

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Visual information processing within the avian ascending tectofugal pathway to the forebrain undergoes essential rearrangements between the mesencephalic tectum opticum and the diencephalic nucleus rotundus. While the outer tectal layers constitute a two-dimensional map of the visual surrounding, nucleus rotundus is characterized by functional domains in which different visual features like movement, color, or luminance are processed in parallel.

Morphological correlates of this reorganization were investigated by means of focal tracer injections into different rotundal or tectal regions in pigeons. Five subtypes of tectorotundal lamina 13 projection neurons were identified. These cellpopulations exhibited a twofold subdifferentiation with their dendrites ramifying within different retinorecipient tectal laminae and their axons projecting to different rotundal subcomponents. Since retinorecipient tectal layers differ in their input from distinct types of retinal ganglion cells, each tecto-rotundal celltype processes different aspects of the visual surrounding. Therefore, the differential input/output connections of the five tectorotundal cellclasses constitute the structural basis for spatially segregated parallel information processing of different stimulus aspects within the tectofugal visual system. As two of five rotundal projecting cellclasses additionally exhibited quantitative shifts along the dorso-ventral extension of the tectum, these data also indicate visual field dependent alterations in information processing for particular visual features.

162 Complex sensory processing in projection neurons of the chick optic tectum: Anatomy, physiology and connectivity

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Several identified cell types in the stratum griseum centrale (SGC) of the chick optic tectum mediate information transfer from the retina to different subdivisions of the thalamic nucleus rotundus. One of these, SGC type I, has been shown to possess specific anatomical, physiological and computational characteristics both *in vivo* and *in vitro*. It is thus possible to delineate the biophysical basics of their physiological response characteristics, namely, the differential response to moving objects compared to moving whole-field patterns.

Type I SGC neurons have large dendritic fields and specialized distal endings (bottlebrush endings, BBE) that receive monosynaptic glutamatergic input from very small retinal ganglion cells. Upon input, a large depolarisation is induced at the BBE which leads to active propagation of excitation along the dendrite. At the soma, excitation from

one dendritic ending is sufficient to activate axonal output. In case of simultaneous excitation from several endings, the input is translated into a chattering response of the soma, the frequency of which is dependent on the depolarization *in vitro* and on aspects of a moving object *in vivo.*

The retinal synapse onto the BBE has peculiar transfer properties. Once activated, the synapse is blocked for additional transmission for a rather long time. The biophysical basis for this phasic response might be provided by local interneurons and has been studied pharmacologically. In addition, the synapse is target to various modulatory input (e.g., nucleus isthmi, nucleus pretectalis) that could alter synaptic time constants and thus response characteristics of the cell. Recent modeling of the system has demonstrated a form-cue invariant response to motion stimuli, a property ususally ascribed to more complex circuitry.

Neurons with characteristics comparable to the avian SGC have been found in the visual midbrain of all amniotes. The general function of this circuitry appears to be the basic analysis of visual scenes with simple but robust mechanisms for the analysis of moving objects. The implications for general visual processing in vertebrates will be discussed.

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Local inhibitory mechanisms control information flow in the mammalian superior colliculus 163

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The mammalian superior colliculus (SC) integrates visual, auditory, and somatosensory information to generate eye movements which control the position of gaze. Visual input from the retina is processed in the superficial layers and conveyed to deeper SC layers in retinotopic coordinates. Particularly in the superficial gray layer, stratum griseum superficiale (SGS), almost every neuron receives retinal input. Because SGS neurons are heavily interconnected, complex local information processing is assumed to occur in this structure. About 50% of SGS neurons are local GABAergic interneurons. They provide feed forward inhibition to neighboring SGS projection cells and other local GABAergic interneurons. As in other structures of the mammalian subcortical visual system, all three types of GABA receptors that have been distinguished so far, $GABA_A$, $GABA_B$, and $GABA_C$ receptors, are highly expressed in SGS. However, the functional roles of the different GABA receptor types are less well understood. In particular, $GABA_C$ receptors have been described only recently, and their significance for SGS information processing awaits further clarification. In order to elucidate their functional role, whole-cell patch-clamp recordings in rat SGS slices were performed and effects of bath-applied $GABA_C$ receptor agonist and antagonists were studied. $GABA_C$ receptor activation by GABA or muscimol decreased electrically evoked postsynaptic activity in GABAergic SGS interneurons. As a result, postsynaptic excitation in projection cells, which are targets of local feed forward inhibition, was increased by $GABA_C$ receptor activation. Suppressive effects achieved by $GABA_C$ receptor activation in interneurons were found early in postnatal SGS development. In contrast, the disinhibitory action of $GABA_C$ receptor activation on SGS projection cells gradually matured postnatally. It appeared to

become adult-like only after local inhibitory circuits had completely developed at the end of the third postnatal week. All this indicates that $GABA_C$ receptors in SGS are selectively expressed by local GABAergic interneurons. Bath-application of agonists does not allow to distinguish between synaptic localization of $GABA_C$ receptors, which would indicate participation in fast information processing, or extrasynaptic localization, pointing towards a modulatory function in the control of the overall amount of GABA released. Therefore, spontaneous activity was induced by application of the potassium channel blocker 4-aminopyridine in SGS to increase the likelihood of inhibitory postsynaptic currents, while blocking excitatory neurotransmission by the unspecific glutamate receptor antagonist kynurenic acid. Although IPSCs under these conditions were mostly mediated by $GABA_A$ receptors, some $GABA_C$ receptor mediated IPSCs were also observed. This indicates that inhibitory neurotransmission in SGS is at least partly mediated by $GABA_C$ receptors. If $GABA_C$ receptors are localized at distinct synaptic sites, it is functionally important to identify the nature of the presynaptic elements at such synapses. In order to test whether $GABA_C$ receptors are activated by a local GABAergic neurons, IPSCs were elicited by focal activation of SGS neurons using UV-induced glutamate photo-uncaging. Because IPSCs evoked this way were always blokked by the selective $GABA_A$ receptor antagonist bicuculline $GABA_C$ receptors seem not activated by local GABAergic circuits. Instead, an external SGS input can be proposed that terminates at synaptic sites characterized by postsynaptic $GABA_C$ receptors.

164 Role of neural synchrony for response selection in the superior colliculus

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Evidence is accumulating that synchronous firing of neurons plays an important role for sensory information processing. Studies on the visual cortex have suggested that correlated activity may serve for the integration of activity into coherent representational states and for the selection of distributed responses for further joint processing. Since population coding is also assumed to be important for sensorimotor transformations carried out in the superior colliculus (SC), we have studied temporal relationships between spatially separate neurons in the tectum. Here, we report experiments carried out in cats and rats that provide evidence for a potential functional relevance of temporal patterning in SC activity.

Using arrays of multiple electrodes, we have recorded from superficial layers of the SC in anesthetized cats. Correlation analysis revealed that neurons in the SC can readily synchronize their activity. Synchronized activity was observed for neurons with overlapping as well as nonoverlapping receptive fields and occurred over distances of several millimeters. Correlograms showed both narrow as well as broad peaks that were centered around 0 ms phase shift. In many cases, the interactions were accompanied by oscillatory components of varying frequency (5-80 Hz). Moreover, the interactions were clearly dependent on the configuration of visual stimuli. Very similar results were ob-

tained by recordings in awake, head-fixed cats, the most notable difference being a higher incidence of γ-oscillations as compared to the anesthetized preparation.

To probe the functional role of collicular synchronization we analysed how electrically evoked saccadic eye movements were affected by varying the temporal relation between microstimulation trains applied to two sites in the SC. Saccades were evoked through microelectrodes positioned in the SC of awake head-fixed cats. As expected, synchronous electrical stimulation led to vector averaging. In contrast, asynchronous stimulation (more than 5ms offset between the pulses of the two stimulation trains) led to vector summation: in this case, the saccades had the same direction as those evoked by synchronous pulse trains, but showed larger variance and approximately double amplitude.

To test whether these findings can be generalized to other species, we have performed recordings from the SC in anesthetized rats. Major features of correlation patterns observed between visually activated cells in the rodent SC were similar to the cat: First, one third to two thirds of nearby cell groups were correlated. Second, correlograms often indicated an oscillatory coupling of neural activity. Third, correlogram peaks were centered around 0 ms phase shift. Fourth, the probability of synchronization increased with the overlap of the RFs and the proximity of the recording sites. Oscillatory neural responses were also recorded from somatosensory neurons driven by vibrissal stimulation.

Taken together, the data suggest that synchrony may be important for processing in the SC and may allow the selection of stimulus-specific motor responses from spatially nondiscrete activity patterns. Since the SC represents a site of multi-modal convergence, targets for orienting responses mediated by the SC might be encoded by assemblies comprising synchronized cells responding in different sensory modalities. Future experiments will be designed to test this possibility.

Computing a neural representation of auditory space in the mammalian superior colliculus 165

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The superior colliculus (SC) transforms different sensory signals into motor commands that control orienting behaviour. The presence within this midbrain nucleus of topographically-aligned visual, auditory and somatosensory maps enables each of the modality-specific cues associated with a common target to be transformed into motor signals that produce orienting movements toward the source of stimulation. The registration of the maps also allows SC neurons to synthesize multisensory inputs according to the relative timing and location of each stimulus. In this study, we have examined the acoustical basis for the map of auditory space in the ferret SC and the behavioural significance of this representation.

The sensory maps in the SC are derived in different ways. Neural maps of visual space and of the body surface arise from topographically ordered afferent projections from their respective receptor surfaces. In contrast, the location of a sound source has to be

computed centrally through the sensitivity of auditory neurons to spatial cues that are present in the sound waves reaching the two ears. These cues include interaural differences in sound level (ILDs) and arrival time (ITDs), as well as spectral cues produced by the way in which the external ear filters the incoming sound. We have investigated the relative contribution of these cues to the spatial receptive fields of deep layer SC neurons, by using virtual acoustic space (VAS) stimuli that replicate over earphones the full set of cues produced by a real free-field stimulus. By using VAS stimuli generated from the animals' own ears, we found that auditory neurons were tuned to locations in the contralateral hemifield that were aligned with the visual responses of superficial layer units recorded in the same electrode penetration. Auditory responses were also examined with VAS stimuli that had ITDs set to an inappropriate value while the remaining cues were allowed to vary naturally. In some cases, this procedure led to changes in peak firing rate or in the size of the receptive fields, but did not affect the topography of the auditory representation and its alignment with the visual map. These findings suggest that sensitivity to ITDs does not contribute to the map of sound azimuth in the mammalian SC and support previous studies in showing that auditory spatial tuning is based on a combination of ILDs and spectral cues.

Another group of ferrets were trained by positive conditioning to localize broadband noise bursts, light flashes from an LED, or both presented together within the frontal hemifield. Both approach-to-target and head orienting responses were consistently more accurate with spatially concordant bimodal stimuli than with either stimulus presented by itself, but less accurate when the visual and auditory stimuli came from different locations. Unilateral inactivation of the SC impaired the localization of all stimuli in the contralateral hemifield, but did not affect the animals' performance on the ipsilateral side. These data suggest that crossmodal map registration in the SC contributes to the ability of animals to localize stimuli that are registered by different sense organs.

166 Cognitive influences on auditory processing in the vertebrate midbrain

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Stimuli in the sensory environment are related to adequate behavioral reactions by means of sensorimotor loops at different levels of processing in the vertebrate brain. Sensory processing at the level of the midbrain (optic tectum in birds and superior colliculus in mammals) is, for example, strongly involved in analyzing the spatial environment. This analysis leads to adequate orienting behavior towards important sources, e.g. in the context of sound localization behavior. On the other hand, the initiation of cognitive processes as attention, which can also be triggered by sensory stimuli, is mainly related to forebrain structures. One of the challenges in current research is to find out how sensorimotor integration at these different levels of processing interact in order to create adaptive behavior in the usually complex arrangement of stimuli in real world situations.

Attention is one of the strongest cognitive influences on the processing of complex sensory environments by selecting important sensory information. We investigated the

effects of attention on sound localization behavior and on the related neural processes at the level of the midbrain in barn owls and rats. Changes in neural activity at the single neuron level were investigated by means of extracellular chronic recording while animals were involved in a spatial cueing paradigm, a behavioral task for measuring effects of attention. In these paradigms, a cue orients spatial attention towards a location, thereby selecting possible target stimuli for detailed sensory analysis.

We found strong effects of attention at the midbrain level (optic tectum in barn owls, superior colliculus in rats). Neuronal activity was increased in neurons that represented the spatial directions selected by attention. In addition, responses of neurons to auditory stimuli from the directions the animal attended to were strongly modulated (increased responses in barn owls, decreased responses in rats). These effects were finally related to facilitated behavioral responses to signals from these directions, i.e. shorter response latency and reduced error rates when the cue stimulus correctly indicated the direction of the following target stimulus. Our recent results indicate a direct correlation between the behavioral and neural effects in rats. In the barn owls we found in addition competing effects in the midbrain activity of attention and another modulatory influence, presumably motor intention, that led to responses of the animal away from the cued direction. The presumed origin of these different types of modulatory influences are forebrain structures.

These results show how cognitive influences from the forebrain can strongly effect the processing at other levels of sensorimotor integration. The effects of such top-down influences can be very important to coordinate different levels of processing in order to create consistent behavior that is useful in a specific behavioral context.

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Primary Culture of Cells from the optic tectum of the Chick: Establishment and characterisation 167

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The optic tectum is a prominent structure in the avian midbrain with a highly laminated architecture. The retina sends its efferents to the layers 2-5 and 7. The majority of tectal efferents arises in layer 13 (Stratum griseum centrale, SGC) and projects to the thalamic nucleus rotundus. The SGC consists of several identified cell types with characteristic morphology, connectivity and physiology. One subtype of these cells, SGC Type I, has been shown to be involved in motion detection.

While many aspects of cellular physiology and computation can be inves-tigated well in a slice, several issues can not be studied due to remaining circuitry in the slice. These limitations can be overcome by complete isolation of the cells in a primary cell culture. SGC neurons have been shown to develop their morphological characteristics without retinal input and might therefore form their typical features even in isolation. A cultured cell could then provide an ideal approach to study the biophysical basis of computation.

Our aim was thus to establish a primary cell culture of tectal neurons. We have delineated and optimised the parameters for cell isolation and growth. With our protocol, we

can isolate neurons in early embryos (~E7) and cultivate the neurons for several weeks. Cells generate extensive neurites and form networks, the characteristics of which are influenced by factors such as the coating of the culture dish. To further characterise these cells, we applied immunohistochemistry with antibodies against neuronal and glial markers. On the basis of these data, we could clearly differentiate neuronal from non-neuronal cells and optimise the preparation accordingly. In addition to these morphological studies, we obtained whole-cell patch recordings of cultivated neurons. We could show neuronal characteristics, including specific responses to somatic current injection found exclusively in SGC type I neurons.

Taken together, tectal neurons can be grown in a primary cell culture where they develop specific neuronal characteristics. This approach will provide an ideal preparation to study cellular computation through localized electrical stimulation and patch-recording. In addition, we will apply molecular methods to investigate the ion channels expressed in the SGC neurons.

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168 Neuronal computation in the avian optic tectum: A compilation of neuron types, their connections and transmitters

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The avian visual midbrain has been investigated for more than a century and is a model system for the analysis of developmental, physiological and computational issues. The major reason for this diverse utilisation is the highly ordered cytoarchitecture of the optic tectum. The avian tectum is a large, multistratified structure with 15 laminae which are easily distinguishable in histological sections. The major input to the tectum comes from the retina, spreads over the surface and terminates in a topographic fashion exclusively in the superficial layers. In contrast, the output of the tectum arises from the deeper layers, most notably the Stratum griseum centrale. This strict separation of visual input and output layers is advantageous for the analysis of cellular computation and sets the avian midbrain apart from the mammalian counterpart, the superior colliculus.

In the literature, a wealth of data is available on developmental, anatomical, physiological and molecular aspects of the avian optic tectum. However, the different research fields are mostly unconnected; e.g., physiological data are very rarely discussed with respect to their cellular basis, and molecular findings are usually not specified well enough to be ascribed to a specific neuron type. This is unfortunate because over the last decade several groups have characterized a variety of different cell types including their morphology and input-output relations. With little effort, many findings could be integrated into this anatomical framework and thus contribute to the delineation of neuronal mechanisms in the tectum. I have therefore compiled data from the literature and from μ own investigations to give a survey of the relevant cellular elements, their connectivity within the tectum and with other brain areas, and their neurotransmitters. In addition, I

have sketched both the general physiology and the molecular characteristics of selected neuronal populations.

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Synaptic depression in motion-sensitive SGC-neurons of the chick optic tectum: Physiological data and modelling 169

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In the avian visual midbrain, neurons of the deep stratum griseum centrale (SGC) neurons have specific response properties: They have large receptive fields, respond best to small moving stimuli, and are not responsive to whole-field motion. In addition, they respond little to a stationary flashing stimulus and they habituate to repeated presentation of the same small moving stimulus. While the morphology and connectivity have been analyzed in detail, the biophysical basis for these response characteristics is not known.

One simple mechanism that would account for part of these response properties is a localized phasic (transient) signal transfer, equivalent to a temporal differentiation, from the retinal ganglion cell axons to the tectal neuron soma. We tested the retino-tectal transmission *in vitro* with double-pulse stimulations to the retinal afferents and whole-cell patch recordings of the SGC neurons. Our data show that the signal transfer is indeed phasic and mediated by synaptic depression at the retino-tectal synapse. Additional experiments on the contribution of GABAergic interneurons in the tectal synaptic layer will be discussed.

We have simulated a simple model of the retino-tectal circuitry. With the transmission parameters derived from the *in vitro* recordings, the model has response properties comparable to the response of SGC neurons *in vivo*, including the differentiation between stationary and moving objects. Moreover, the model shows a form-cue invariant response to motion, a property that is usually thought to involve complex processing.

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Separation of Ascending and Descending Tectal Projections within the Tectofugal Pathway of the Pigeon 170

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The optic tectum of vertebrates is an essential relay station for the generation of visually elicited behavior. In agreement with its functional role, the laminated tectum is characte-

rized by a basic set of connections that comprise topographically ordered input from the eyes and output that reaches the premotor hindbrain regions. In birds, the deep efferent layer 13 is the exclusive source of the ascending projection to the thalamic nucleus rotundus1, 2, 4, 5. Since this layer also contributes substantially to the descending projections, it was suggested that individual cells might contribute to both pathways6 as it has been shown for amphibia3.

In order to identify cells with ascending and descending axon collaterals, we performed injections of Choleratoxin subunit B (CtB; Sigma) unilaterally into either the lateral tectobulbar or the medial tectopontine fiber tracts of adult pigeons. Additionally, all animals received bilateral injections of Texas Red dextran amine (TDA, Molecular Probes) into the central and dorsal diencephalon to label the ascending tectal cells. After 5 days, the animals were perfused, the brains were cryosectioned and an immunohistochemical detection of CtB was performed.

Irrespective of the tegmental injection site, large numbers of CtB-immunoreactive cellbodies were labeled in both tectal hemispheres. After lateral tegmental injections, retrograde labeling within the tectum concentrated in the ipsilateral tectum whereby cellbodies formed two rows. One group of cells was located within layers 6-10. Besides, a large number of multipolar layer 13 neurons were labeled. After medial tegmental injections, labeled cells were localised within the deep tectal layers 12-15 with a higher proportion of labeled cells within the contralateral tectum. Moreover, in all preparates high numbers of TDA labeled tectal cells were bilaterally detected within layer 13. In all animals that exhibited substantial labeling of both, the ascending (TDA) as well as the descending tectal projections (CtB), the number of double labeled cells was determined. This analysis revealed that the proportion of double labeled cells (relative to the number of CtB filled cells) was extremely low with 0.38% (n=7; SD=0.55) for medial tegmental and with 0.26% (n=4; SD=0.12) for lateral tegmental injections. Thus, our data demonstrated a virtually complete separation of ascending and descending tectal cell groups.

The present study, in combination with an earlier study on the differentiation of the ascending tectal output5, provides evidence for a highly differentiated projection pattern within lamina 13, the major tectal output layer. Our data facilitate the view, that the tectum processes visual input independently within multiple ascending and descending visual information streams.

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171 Auditory Attention and Spatial Selection Behaviour Effect the Neuronal Activity in the Superior Colliculus in Rats.

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Attention can be directed to a location thereby selecting objects for detailed sensory analysis. A task to quantify such orienting of attention was devised for the visual sy-

stem: with a subject fixating centrally, a peripheral cue is presented followed by a peripheral target (Posner, M.I., 1980, Q.J.Exp.Psychol. 32:3). In 80% of the trials, the (valid) cue indicates the side of the subsequent target, in the remaining 20%, the (invalid) cue indicates the side opposite to the target.

We recorded neural activity in six awake rats performing an auditory version of the cueing paradigm. For cue and target two white noise pulses separated by four different randomized delay periods were used (100, 300, 500, and 700 ms). The duration of the cue was 100 ms. The target was presented until the animal reacted, but not longer than 300 ms. The effect of target detectability was examined by systematically reducing the sound pressure level of the target. In each animal six electrodes were implanted in auditory responsive areas of the superior colliculus (SC). Recorded data was separated in single and muli unit activity cluster. Further analysis was performed on modulation indices (MI), calculated as the contrast of mean activities between the valid and invalid stimulus configurations.

Data analysis showed that activity in the SC contralateral to the cued side was increased compared to the activity ipsilateral to the cue during the delay period. Cueing, therefore, shifted the balance of the two SC hemispheres towards the attended hemifield of auditory space. Activity during target presentation, however, was decreased in the valid stimulus configuration compared to the invalid stimulus configuration. Thus, a selective modulation of neurons based on the spatial orienting of attention was observed, indicating an influence of attention already at the midbrain level.

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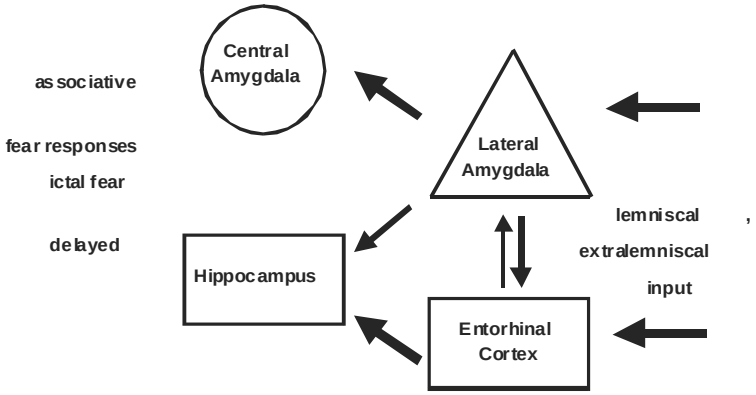
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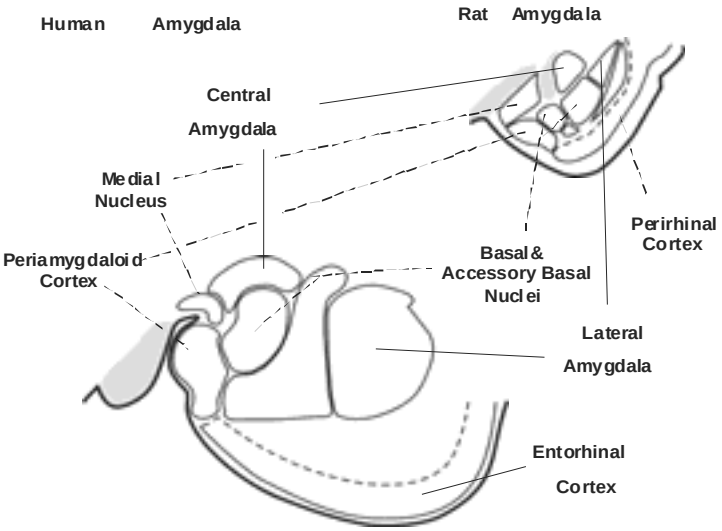
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Cortex
Basal &
Accessory Basal
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Perirhinal
Cortex
Lateral
Amygdala
Entorhinal
Cortex

Introductory Remarks to Symposium 19

Function and dysfunction of the amygdala: fear and epilepsy

Deniz M. Yilmazer-Hanke and Oliver Stork

Amygdala research currently focusses on two related and clinically relevant aspects of amygdalar function and dysfunction, fear and epilepsy. These appear to interact in human and experimental temporal lobe epilepsy (TLE), as intracerebral recordings have suggested a contribution of the amygdala not only to generation and propagation of focal seizures but also to the elicitation of ictal fear during temporal lobe seizure activity. The detailed morphological and physiological characterization of the amygdala's extrinsic and intrinsic connectivity in recent years now allows to approach the mechanisms underlying these (dys-)functions (Pitkänen et al., 1997; 2000). In essence, it is believed that the basolateral complex of the amygdala acts as a „sensory gate"" and integrates exteroceptive and interoceptive sensory information of different modalities, which is then relayed via the central nucleus to vegetative centres in the hypothalamus and brainstem. Much physiological work has focussed on the glutamatergic transmission at sensory afferents to the lateral amygdala, such as the NMDA-receptor dependent and long term potentiation-like enhancement of neural activity after fear conditioning training, and the metabotropic glutamate receptor-dependent epileptogenesis (McKernann and Shinnick-Gallagher, 1997; Neugebauer et al., 1997). The importance of GABAergic interneurons in the basolateral complex, that regulate the activity of pyramidal projection neurons by feed-forward and feed-back inhibition, is also widely appreciated. Evidence suggest that, in fact, a loss of perisomatic inhibition in the amygdala may relate to the enhanced excitability of glutamatergic projection neurons in human TLE. Various subpopulations of GABAergic and peptidergic neurons in the basolateral complex and central amygdala further play important and specific roles in the regulation of stress and fear responses. The prominent monoaminergic innervation of the amygdala has been implicated in the control of many of these neurons. Amygdala-hippocampal interactions are particularly important for cognitive aspects of fear conditioning, as well as stress-related effects on hippocampal synaptic plasticity and hippocampus-dependent memory consolidation (Akirav and Richter-Levin, 1999). On a molecular level it has become evident that the activation of various protein-kinase pathways leads to an activation of gene-expression from cyclicAMP-responsive elements and subsequent induction of immediate early gene transcription factors, as well as signal transduction and structural reorganisation factors in the amygdala.

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172 Cellular and structural alterations leading to increased excitability of the amygdala in human temporal lobe epilepsy

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The amygdala has attracted particular attention in the recent years because of its importance for focal seizures in human and experimental temporal lobe epilepsy (TLE). Many common symptoms and signs in the course of temporal lobe seizures result from amygdala activation as revealed by intracerebral EEG recordings in TLE patients, and are accompanied by amygdalasclerosis as a histopathological correlate. However, little is known on the cellular mechanisms or structural reorganisation patterns, that lead to an increased excitability of the amygdala in TLE .

In order to characterize the cellular pathology of the amygdala in human TLE, cytoarchitectonical, myelin and immunohistochemical glial fibrillary aidic protein (GFAP) stains as well as intracellular Lucifer Yellow (LY) injections were applied to 3-4 autopsy controls and 16-17 surgical specimens obtained from TLE patients. In an electronmicroscopical study, interneurons containing parvalbumin and GABA decarboxylase were analyzed in the lateral nucleus of nine TLE patients as well as in two monkeys (cases, macaca mulatta) and two Wistar rats.

Major histopathological alterations of TLE patients included neuronal cell loss accompanied by fibrillary gliosis in all nuclei of the basolateral complex, which is the major port of entry to the amygdala. Moreover, there was a reduction of neuronal soma size and an increase in the maximum spine density of excitatory projection neurons of the basolateral complex, identified as spiny, modified pyramidal neurons, that are known to contribute both to extraamygdaloid and intraamygdaloid-internuclear projections. The ultrastructural study of GABAergic circuits in the basolateral complex of the amygdala revealed a remarkable loss of symmetrical inhibitory synapses on the somata of amygdaloid projection neurons in human TLE, although the somata and dendrites of parvalbumin-containing neurons received excitatory synaptic input as shown in the rat amygdala. In particular, the GABAergic parvalbumin-containing interneurons are able to perform a powerful feedback inhibition of the basolateral complex, because these neurons receive excitatory synaptic input from recurrent axon collaterals of amygdaloid projection neurons and in turn form inhibitory synapses on the somata and axon initial segments of projection neurons. It is concluded that loss of perisomatic inhibition, that is formed by parvalbumin positive basket and chandelier cells under healthy conditions, leads to a reduced inhibition of glutamatergic projection neurons of the lateral nucleus of

amygdala, that projects to the basal and granular nuclei of the amygdala as well as to the entorhinal cortex. Altogether, the structural reoganization patterns found in the amygdala are in a position to induce an enhanced activity of glutamatergic projection neurons resulting in seizure generation, propagation and chronification in human TLE.

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The amygdala in the maintenance of learned fear 173

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Fear conditioning, a behavioral model of fear learning and cue-related anxiety, causes enhanced neuronal transmission in the thalamic to lateral amygdala pathway. Here evidence is presented suggesting that NMDA receptors and L-type calcium channels are altered in the maintenance phase of fear memory. In these experiments fear potentiated startle is measured in animals in which tone and foot shock are presented in paired or unpaired paradigms. Forty-eight hours after training and 24 hours after testing lateral amygdala neurons are analyzed with whole cell recordings.

In the lateral amygdala fear conditioning is associated with an enduring increase in synaptic strength mediated through non-NMDA receptors and with a reduction in paired-pulse facilitation (PPF) reflecting an increased probability of neurotransmitter release. In contrast, NMDA receptor-mediated transmission at the thalamic to lateral amygdala pathway is not facilitated after fear conditioning although probability of transmitter release is enhanced. Rather, the EC50 for NMDA receptor-mediated current is shifted 3-4 fold to the right in fear-conditioned animals, suggesting a postsynaptic alteration in NMDA receptors in the maintenance phase of long-term fear memory. Furthermore, the ability of ifenprodil, a selective antagonist of the NR2B subunit, to block an NMDA receptor-mediated EPSC is reduced in lateral amygdala neurons from fear-conditioned animals, suggesting a reduction in NR 2B subunit-containing receptors at thalamo-lateral amygdala synapses. In addition, western blots show a reduction in protein expression of phospho-NR1, NR2A, and NR2B subunits in amygdalae from fear-conditioned but not context control animals.

Although not engaged in control animals, L-type calcium channels are involved in synaptic transmission in fear memory. Nimodipine (Nim, 1.5-20 mg/kg), an L-type calcium channel blocker, administered intraperitoneally, reduced fear-potentiated startle but not baseline startle in a dose-dependent manner. In neurons from naïve control animals, Nim had no effect on EPSC amplitude or PPF but, in slices from fear-conditioned rats, Nim reduced EPSC amplitude suggesting the recruitment of L-type calcium channels within the fear-conditioning circuitry. Nim increased PPF in slices from fear-conditioned animals suggesting that L-type calcium channels may contribute to increased probability of release in fear conditioning. In slices from unpaired animals, Nim decreased synaptic transmission but had little effect on PPF suggesting that stress or contextual fear learning may induce L-type channel activity in fear conditioned and unpaired control animal groups. We also analyzed the protein for the a1C and a1D L-

type calcium channels isolated from the amygdala and found that a1C protein was significantly increased in fear conditioned animals.

Together these findings suggest: 1) that down regulation of the NMDA receptor may confer protection from potential excitotoxicity of unchecked NMDA receptor recruitment resulting from induction and consolidation of fear memories, 2) that this reduction in NMDA current and protein may contribute to mechanisms underlying the maintenance of long-term fear memory and allow "persistence of the capacity" to reactivate amygdala pathways in NMDA receptor-dependent fear memories and 3) that L-type calcium channels play a role in the amygdala in cued fear conditioning and have important implications in the treatment of anxiety and in emotional learning and plasticity.
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174 Effects of amygdaloid kindling on post-ictal plasticity in the lateral nucleus of the amygdala

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Memory impairment is one of the most common consequences of seizures, and long-term potentiation (LTP) has been conceptualized as a reflection of mechanisms underlying memory formation. The aim of the study was the analysis of synaptic responses, short-term plasticity and LTP in the lateral nucleus of the amygdala (LA) of kindled rats. The kindling phenomen is considered as a useful experimental animal model of temporal lobe epilepsy (TLE). Animals with electrodes implanted into the left basolateral nucleus were stimulated until 7 and 15 consecutive stage V seizures, respectively. Combined horizontal brain slices, prepared 48 h after the last stage V seizures, were used for the extracellular recordings. The recording as well as the bipolar stimulation electrode was positioned within the LA. The results showed that the electrode implantation in controls itself reduced the basal synaptic transmission in the LA. As it can be expected, the left hemisphere (locus of electrode implantation) was more impaired than the contralateral one. However, LTP induced at the right lateral amygdala was significantly stronger than that recorded at the left side even in non-operated, untreated control animals. These results might have some relation to the hypothesis of a right hemisphere dominance for negative emotions. To assess whether kindling alters the presynaptic transmitter release in comparison to age-matched operated controls, paired pulse facilitation (PPF) was tested. The kindling procedure caused a significant decrease in the paired pulse ratio after 15 stage V seizures, indicating an enhancement of release probability. In addition, kindling enhanced synaptic responses to single stimuli (upward shift of input/output curves). In contrast, the high frequency stimulation-induced LTP was significantly impaired in kindled animals. A reduced LTP after kindling was also found in the CA1 region of the hippocampus. These results show correlations to the data of LTP experiments performed in human epileptic tissue. A reduced neuroplasticity may also be one of the reasons of emotional disorders observed in some TLE patients, such as depressive and anxiety-related symptoms. The mechanisms underlying impaired amygdaloid and hippocampal long-term plasticity in kindled rats remain to be elucida-

ted. They may be related to kindling-induced cell loss that results from these processes. At least after 15 consecutive stage V seizures the reduced LTP was associated with axonal degeneration in the left LA. The neuronal density within the LA was also significantly lower in kindled rats compared to implanted ones. Further experiments are needed to clarify to which extent functional changes after kindling may explain the impaired neuroplasticity in the LA.

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Amygdalo-hippocampal connectivity and its activation during fear conditioning 175

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Several studies implicate a role for the amygdala in the processing of emotional memories which might partially occur in the connections between the amygdala and the hippocampal and parahippocampal areas. The present study was designed to determine if the pathway from the amygdala to the entorhinal cortex becomes activated during acquisition of fear-conditioning. First, the retrograde tracer Fluoro-Gold (FG) was iontophoresed into the entorhinal cortex in rats. Following habituation, conditioned animals received a single tone-footshock pairing, and controls received another habituation session. Then the brains were processed for double-immunohistochemistry against c-Fos and FG or glutamate decarboxylase (GAD67). Labeled neurons were plotted and their numbers calculated in the various divisions of the lateral and basal nuclei of the amygdala. In conditioned animals the number of c-Fos positive nuclei increased in dorsolateral division (194% of that in handled controls, $p<0.05$) > medial division (160%, $p<0.05$) > ventrolateral division (144%, $p<0.05$) of the lateral nucleus. Additionally, in the medial division of the lateral nucleus, the percentage of c-Fos/FG double-labeled neurons was significantly higher in the conditioned animals compared to the control group (6.3% ± 2.5% vs 2.2% ± 1.4%, $p< 0.05$). Only a very few GAD67-positive interneurons expressed c-Fos. These data indicate that a part of the amygdalo-entorhinal pathway is activated during acquisition of fear-conditioning. These data support the idea that emotionally relevant sensory information in the lateral nucleus can influence information processing in the hippocampal and parahippocampal areas via the amygdalo-entorhinal pathway.

Monoaminergic afferents and their targets in the rat amygdala: Implications for stress and fear responses 176

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The amygdala possesses a dense, heterogeneous innervation by monoaminergic afferents. Behavioural and physiological experiments, carried out primarily in the rat, indicate that these afferents play a pivotal role in orchestrating stress-induced autonomous,

behavioural and affective changes, and have additionally implicated peptidergic, especially corticotropin-releasing-factor(CRF)-producing amygdaloid systems in generating anxiety and stress responses. It has been suggested that monoaminergic-peptidergic interactions in the amygdala underly many stress responses. Direct morphological evidence for such interactions is lacking.

We performed studies to provide knowledge about targets of monoaminergic afferents in the rat amygdala. Contacts of monoaminergic afferents with neurons of different amygdaloid nuclei were analyzed and quantified, target neurons were morphologically and neurochemically characterized. *Serotonergic* fibers were dense in all but the lateral central nucleus (CeL). Synaptic contacts were rare. *Adrenergic* and *noradrenergic* afferents to the medial central nucleus (CeM) and adjacent areas, presumably derived from medullary centers, formed mostly asymmetric synapses on distal processes of target neurons, some of which were NPY-immunoreactive (ir). Most, if not all, CeM neurons were glutamate decarboxylase (GAD) mRNA-reactive, and since many presumably project to brainstem centers, these connections may represent long feedback loops with medullary visceral centers. *Noradrenergic* afferents to laterobasal nuclei, presumably arising from the Locus coeruleus, formed predominantly symmetric synapses on peripheral dendrites of neurons, some of which were identified as NPY- or Somatostatin-ir interneurons. *Dopaminergic* innervation was dense in intercalated and in the basolateral nuclei (BL). GABAergic neurons of intercalated nuclei expressed D1-receptor (D1R) mRNA and were often perisomatically innervated, indicating a strong effect of dopamine on the output of these neurons. In the BL, predominantly projection neurons, most likely expressing the D1R, were synaptically contacted. In the CeL, where other monoaminergic fibers were scarce, dense dopaminergic fiber plexus with high synaptic density overlapped with clusters of CRF-ir neuronal cell bodies. Ultrastructurally, individual neuronal cell bodies displayed intense perisomatic dopaminergic synaptic innervation. Double labeling showed that only a minority of the enkephalin or somatostatin-ir neurons but practically all CRF-ir neurons were perisomatically contacted, indicating that dopaminergic innervation exerts strong control specifically on the CeL-CRF neurons. Correlative *in situ* hybridization suggested that CeL neurons express D2R.

The findings are a basis for interpretation of the results of pharmacological and behavioural studies into monoamine functions in the different amygdaloid nuclei. Of particular interest for elucidating the role of monoaminergic-peptidergic interactions in stress and anxiety responses is the fact that the CeL-CRF neurons are under dopaminergic control, possibly via the D2R. In the striatum, D2R-mediated transmission leads to decreased peptide expression in target neurons. It is conceivable that the dopaminergic innervation serves as an inhibitory regulator of CRF-expression in the CeL, thereby modulating CRF-induced stress-evoked and fear-related responses.(Supported by the DFG, AS 89/2-1)

Emotional modulation of memory - Stress modulation of plasticity in the hippocampus and amygdala 177

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Stress impairs hippocampal long-term potentiation (LTP), a model of synaptic plasticity that is assumed to underlie memory formation. In the amygdala, little is known about the effects of stress on LTP, or about its longevity. Here we assessed the ability of entorhinal cortex (EC) stimulation to induce late-phase LTP (L-LTP) simultaneously in the basal amygdaloid nucleus (B) and in the dentate gyrus (DG) of freely behaving rats. Once established, we investigated the effects of novel vs. familiar inescapable moderate stressful experiences on LTP in both structures. Results show that B, like DG, sustained LTP for 7 days. Furthermore, a single exposure to a moderate stress facilitated LTP in B but did not affect DG LTP. In contrast, stress re-exposure inhibited LTP both in B and DG. Behaviorally, animals exhibited a higher immobility when re-exposed to the moderate stressor as compared to a single/first exposure. These data support a role for B in memory storage. They also suggest that novel stress may be a learning-supporting situation whereas familiar stress may induce a depressive-like status in which learning and memory are hampered. Together, the results support a differential involvement of the amygdala and hippocampus in memory formation and storage depending on the emotional characteristics of the experience. The results are discussed in relation to their potential relevance to anxiety disorders.

Molecular mechanisms of fear memory: Gene expression and transgenic approaches 178

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The amygdala is a key structure for the regulation of emotional state and the integration of sensory and affective information, and comprises a critical site of synaptic plasticity during Pavlovian fear conditioning. We investigated these functions on a molecular level in combination with behavioural and *in vivo* electrophysiological approaches. First, changes of gene expression in the basolateral complex of the amygdala that are associated with the formation of Pavlovian fear memory were studied. Polymerase chain reaction-based expression analysis revealed the differential regulation of several signal transduction (induction of aldehyde reductase, phosphodiesterase, somatostatin; decrease in glutamate decarboxylase (GAD)65, neuropeptideY) and structural reorganisation factors (induction of the neural cell adhesion molecules NCAM and neuroligin, the E2 ubiquitin conjugating enzyme and E3 ubiquitin ligase Praja1) during consolidation of fear memory. Stress-related and memory-specific effects could be distinguished in different behavioural training groups. Two of the identified molecular factors were further investigated with a behavioural genetics approach: 1) NCAM null mutant mice displayed an increased anxiety-like behaviour and hyper-responsiveness to anxiolytic 5-

HT1A agonism but deficits in formation of fear memory. Innate emotional behaviour, but not fear memory formation could be recovered through a transgenic re-expression of the 180kD isoform of NCAM in postmigratory forebrain neurons. It is suggested that NCAM may be involved in both serotonergic processes controlling anxiety-like behaviour and in morphological re-organisation processes in the amygdala involved in the formation of Pavlovian fear memory. 2) GAD65 deficient mice, which postnatally develop a GABA deficit in the amygdala and hypothalamus, showed increased anxiety-like and conditioned fear behaviour as well as insensitivity to anxiolytic diazepam. This is in good agreement with the reduced expression of GAD65 after fear memory consolidation and a reduction of extra cellular GABA levels in the amygdala during conditioned fear retrieval. GABAergic interneurons in the amygdala are thought to play an important role in the control and synchronisation of neural activity in the amygdala and the modification of GAD65 expression following fear conditioning training may strongly affect the neural activity patterns in this brain area. In fact, we could demonstrate an increase of rhythmic θ oscillations in the mouse amygdala and their synchronisation with hippocampal rhythms during the retrieval of conditioned fear. Supported by the Deutsche Forschungsgemeischaft (DFG).

179 Functional and molecular characterization of neurons in the human lateral amygdala

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The amygdaloid complex is one component of the temporal lobe that may play an important role in the pathogenesis of human temporal lobe epilepsy (TLE). In this study we used the patch-clamp technique to investigate functional properties of neurons in the lateral nucleus of the amygdala. Acute amygdaloid brain slices were obtained from surgical specimens of patients suffering from pharmaco-resistant TLE. In order to identify neuronal subtypes, lucifer yellow or biocytin was injected into the recorded cells via the patch pipette. Together with functional criteria, subsequent morphological assessment allowed to distinguish between projection neurons and local interneurons. Alternatively, interneurons were identified by localizing transcripts encoding neuropeptides and calcium-binding proteins (NPY, somatostatin, CCK, calbindin, parvalbumin) in electrophysiologically characterized neurons with single cell RT-PCR. The excitability of neurons is influenced by the opening of G-protein coupled inward rectifier potassium channels (Kir3 family). Closed at rest, these channels are activated via $G_{i/o}$-coupled metabotropic receptors. In the human lateral amygdala, functional expression of Kir3 channels was evidenced by applying the $GABA_B$ agonist, baclofen, or group II and group III mGluR agonists (DCG-IV and L-AP4, respectively). Kir currents were separated in the presence of antagonists blocking voltage-gated potassium and sodium channels. Interestingly, Kir3 currents could be activated in most of the projection neurons while interneurons usually lacked these currents. Kir3 channels are assembled from four different subunits, Kir3.1-4. In order to identify the Kir3 subunits that are expressed by

human amygdaloid neurons, single-cell RT-PCR was performed after functional analysis. Most cells co-expressed the subunits Kir3.1 and Kir3.2. Since mGluR functioning in amygdaloid neurons is modified in experimental epilepsy, our data suggest that altered gating of Kir channels adds to the dysregulation underlying seizure generation in human TLE.

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Potentiation of amygdaloid and hippocampal auditory evoked potentials in a discriminatory fear-conditioning task as a function of context and tone pattern 180

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According to the local memory storage hypothesis, information about the tone-shock association in an auditory fear-conditioning paradigm is stored in synapses within the lateral amygdala (LA). Thus, fear-conditioning induced potentiation of auditory-evoked potentials (AEPs) in response to a conditioned stimulus (CS+, a series of short lasting tones; patterned tone) has been interpreted as *in vivo* correlate of amygdaloid synaptic plasticity. Here, we re-examine the specificity of potentiation of AEPs in terms of (1) local confinement to the LA, (2) parameters of CS+ and (3) influence of context, using a discriminatory fear-conditioning paradigm. Adult male C57BL/6J mice were implanted with recording electrodes aimed at the LA, the CA1 region of the hippocampus and the neck muscles for simultaneous recordings of AEPs and startle responses. In a neutral context, AEPs within LA and CA1 as well as startle and freezing responses to the CS+ were significantly potentiated following conditioning, as compared to pre-conditioning values and responses to a neutral stimulus (CSn; tone of different frequency). Potentiation was only evident if CS+ was presented as a uniform series, but not if presented mixed with CSn. Accordingly, mice failed to show intensified freezing to a patterned tone if a single lasting tone of the same frequency served as CS+. Both CA1 and LA AEPs were potentiated in response to CSn, if presented in the conditioning context. These findings demonstrate that (1) potentiation of AEPs is not restricted to the LA, (2) both tone frequency and pattern of tone presentation are essential for proper CS+ recognition, and (3) contextual memory leads to a general potentiation of AEPs.

181 Selective blocking of n-type calcium channels of hippocampal neurons by antiepileptic drug levetiracetam

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Intracellular mechanisms of the anti-seizure-activity of the antiepileptic drug levetiracetam (LEV) were studied in patch-clamp whole-cell experiments. We tested the sensitivity of different types of voltage-operated calcium channels from isolated rat CA1 pyramidal neurons to LEV action. For the separation of calcium channel subtypes, their selective blockers were used: nifedipine, ω-Conotoxin-GVIA, ω-Agatoxin and ω-Conotoxin-MVIIC for L-, N-, P- and Q-types respectively. HVA Ca^{2+} currents were obtained by depolarization of membrane from the holding potential from –70 mV to depolarizing test potentials –10mV or 0 mV for 50ms, and the LVA current from –80 mV to -45mV. In control cells L-type current accounted for 25%, N-type for 45.5%, P-type for 16.8% and Q-type for 9.4%. LEV application partly and irreversibly inhibited the evoked currents in all tested cells. IC50 for the inhibition by LEV of HVA calcium channels was 14.7μM. The maximum inhibition was observed at 200μM and was about 18%. Application of 200μM LEV in the presence of the corresponding selective blokkers further reduced the calcium current amplitude by 17.0%, 15.6% and 17.4% for L-, P- and Q-types respectively. However, LEV was ineffective in the presence of the selective blocker of N-type calcium channels. We did not observed any effect of LEV on LVA Ca^{2+} channels. The present study indicates that LEV selectively blocks the activity of N-type calcium channels of CA1 pyramidal hippocampal neurons with 37% maximal inhibitory efficacy. The difference in blocking action of ω- Conotoxin-GVIA and LEV in N-type channel inhibition is discussed.

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182 Synchronization of amygdalar and hippocampal θ oscillations during retrieval of Pavlovian fear memory

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Much evidence from human and animal studies indicating that interactions between the amygdala and the hippocampus are particularly important in modulation of long-term memory formation, depending on the emotional relevance of the stored information [1,2], e.g. in Pavlovian fear memory. It is widely accepted that cooperativity in the involved neuronal networks can relate to synaptic modifications and memory processes, but the significance for the behaving animal is often poorly understood [3].

In this study, electrical activity was simultaneously recorded in the lateral amygdala (LA) and the CA1 area of the hippocampus in freely-behaving fear-conditioned mice, and patterns of bio-electrical activity were related to defensive behavior (e.g. freezing) evoked by conditioned and indifferent sensory stimuli.

In the present study evidence is shown for an increase of rhythmically synchronized activity at θ frequencies (4-8 Hz) between LA and CA1 upon fear conditioning, that results from an entrainment of amygdaloid activity into hippocampal θ waves and becomes significant during confrontation with conditioned fear stimuli and expression of freezing behavior. The synchronization of neuronal activity at θ range in LA/CA1 circuits seems to represent a neuronal correlate of conditioned fear, apt to improve neuronal communication and/or the segregation of neuronal assemblies during memory retrieval.

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Characterization of somatostatin effects in the rat lateral amygdala

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While there is evidence for an inhibitory role of somatostatin (SST) in seizures and epileptogenesis in the limbic system, the underlying cellular mechanisms are not well understood. Therefore, we investigated postsynaptic effects of SST in neurones of the rat lateral amygdala (LA) using the whole cell patch clamp technique in an *in vitro* slice preparation.

Approximately 95 % of presumed projection neurones developed an outward current on application of 1 µm SST at a holding potential of -70 mV, amounting to 44.7 ± 7.4 pA (n = 8). The induced current was not affected by 1 µm TTX (48.8 ± 7.7 pA, n = 7). Basic properties included inward rectification, dependence on extracellular K^+ and reduction by 100 µm Ba^{2+} applied externally (16.5 ± 2.4 pA, n = 9). Half-maximal effects were obtained at 171.0 nM with a Hill-coefficient of 1.7.

Responses to SST (500 nM) were reduced to 23.8 ± 6.2 % (n = 5) by a SSTR-2 antagonist ([H-Fpa-cyclo[DCys-Pal-D-Trp-Lys-Tle-Cys]-Nal-NH_2, 2 µm).

Involvement of G-proteins was indicated by blockade of action of SST during intracellular presence of GTP-γ-S (100 µm, n = 6) or GDP-β-S (2 mM, n = 5), respectively.

Under current-clamp conditions, SST (1 µm) impaired cell excitability by induction of a membrane hyperpolarization from the resting membrane potential averaging to -8.0 ± 1.4 mV (n = 9), associated with a decrease in apparent input resistance from 588.9 ± 61.7 MOhm to 372.2 ± 34.1 MOhm (n = 9).

We conclude that SST activates a G-protein activated inward rectifying K^+ conductance in LA projection neurones, leading to membrane hyperpolarization and inhibition of these neurones.

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184 Short- and long-term adaptation to aversive situations in C57BL/6JOlaHsd mice

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In the recent years, auditory fear conditioning has emerged as a leading paradigm for studying cellular mechanisms underlying the extinction of aversive memories. In that task, animals form an association between a tone and a mild electric shock. On re-exposure to the tone in absence of reinforcement, animals extinguish their fear reaction both acutely during tone presentation (short-term extinction) and over the course of repeated tone presentations (long-term extinction). The fear reaction to the tone might consist of two different components, a plasticity component (related to the association between tone and aversive stimulus) and an emotionality component (related to the digestion of the electric shock; sensitisation). We tried to dissociate these two components by comparing short-term and long-term extinction of conditioned (tone-shock association) and sensitised (shock only) C57BL/6JOlaHsd mice. It turned out that for short-term extinction the aversiveness of the tone (modulated by shocks of different intensity) was reflected by the decay constant of the extinction curve, while the initial freezing response estimated was similar for both conditioned and sensitised mice. On repeated tone presentation 6 days later, the initial freezing reaction was strongly reduced in sensitised mice, independently from the intensity of the sensitisation protocol. The freezing response of conditioned mice dropped significantly compared to the first tone presentation and failed to mirror differences in the conditioning intensity (modulated by number of tone-shock pairings and intensity of the shock) anymore. Our data indicate that adaptation to aversive tones follows similar patterns in sensitised and conditioned mice. We hypothesise that the changes in freezing behaviour reflects modulations of unspecific emotional components of the conditioning procedure (pain, stress) rather than of the specific association between tone and shock.

Comparative immunolabeling for corticotropin-releasing-factor(CRF) and monoaminergic afferents in mouse and rat amygdaloid complex

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Generation of knockout and transgenic mouse models has made it possible to manipulate individual components of neuromodulator systems *in vivo* in order to elucidate their role in specific brain functions, for instance in orchestrating the various aspects of the central stress response. For analysis of CNS alterations in mouse models, and for planning and interpreting behavioural and pharmacological studies, detailed knowlegde of the normal morphology of the different systems is a necessary prerequisite. To date, due to a lack of appropriate data from mouse brain, most interpretations are based on morphological findings in rat brain, although interspecies differences are often evident. To provide a basis for investigations into the role of monaminergic and peptidergic systems in amygdala-mediated stress and fear responses in genetically altered mice, a detailed correlative analysis of the distribution of serotonergic and catecholaminergic afferents and of CRF-immunoreactive(ir) elements in amygdaloid nuclei of C57Bl/6 mice was carried out in the present study.

Serial vibratome sections of perfusion-fixed mouse brains were reacted for serotonin (5HT)-, serotonin-transporter(SERT)-, tyrosine hydroxylase (TH)-, and CRF-immunoreactivity. The distribution pattern of 5HT- and SERT-ir fibers was identical, but immunolabeling intensity and fiber density were higher in SERT-immunoreactions. Serotonergic fiber density was low in the lateral central nucleus (CeL), moderate in the lateral capsular central nucleus (CeLc), and dense in other nuclei. In the CeL, TH-ir fibers formed particularly dense terminal plexus. TH-ir fibers were scarce in the CeLc and in the lateral nucleus, dense in basal nuclei, and moderately dense in other nuclei. CRF-immunoreactions labeled particularly dense fiber plexus in the CeLc. Less dense, punctate staining was present in the CeL. CRF-ir cell bodies were not clearly identified.

While the serotonergic and catecholaminergic innervation of the rat and mouse amygdala appear similar, CRF-ir elements in the central nucleus are differently distributed. In the rat, CRF-ir neurons and fibers are localized almost exclusively in the medial CeL, where they are intermingled with dopaminergic terminals while serotonergic fibers are scarce. In the mouse, densest CRF-ir fiber plexus overlap with serotonergic afferents in the CeLc. CRF-immunoreactivity in the CeL is less intense than in the rat, and CRF-ir cell bodies are not readily recognized immunocytochemically. Experiments in rats suggest that monoaminergic, particularly dopaminergic/CRF-interactions play an important role in amygdala-mediated stress and anxiety reactions. The observed interspecies differences indicate that interactions of the neuromodulators in the mouse may be differently organized than in the rat. This should be taken into account for interpretation of findings in genetically altered mice.

Supported by the Deutsche Forschungsgemeinschaft, SFB 581

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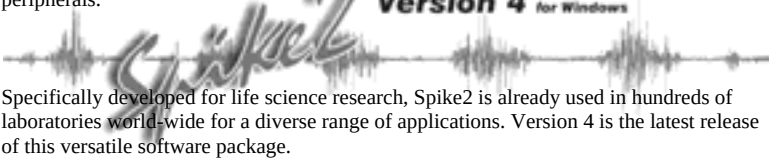

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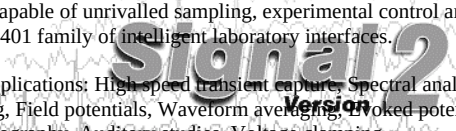

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Introductory Remarks to Symposium 20

Transsynaptic signalling at central glutamatergic synapses

Volkmar Leßmann and Kurt Gottmann

Central synapses are intercellular junctions dedicated to fast presynaptic neurotransmitter release and to sensitive postsynaptic transmitter receptiveness. Exact alignment of pre- and postsynaptic specializations is fundamental to synaptic function. Transsynaptic signalling in both anterograde and retrograde direction is thought to control and regulate synaptic organization and plasticity. One class of molecular mechanisms involves the release of protein factors, binding to their cognate receptors and triggering of downstream signalling cascades. Prominent examples for such a mechanism are neurotrophins, in particular brain-derived neurotrophic factor (BDNF).

Other mechanisms consist of transsynaptic interaction of membrane-bound molecules in a ligand-receptor mode triggering asymmetric signal transduction pathways. Ephrins and eph receptors are a particularly well studied, prototypic pair of this type. Symmetric transsynaptic interactions are mediated by classical cell adhesion molecules, such as N-cadherin. These adhesion molecules bind to each other in a homophilic way, giving rise to symmetric signalling in the pre- and postsynaptic neuron. Last not least, glial cells have recently been found to release factors that are essential for the formation and maintenance of functional synapses, thus supporting the emerging concept of a tripartite synapse.

This symposium concentrates on glutamatergic synapses, because transsynaptic signalling in long-term synaptic plasticity has been a major focus of neurobiological research at these excitatory central synapses. Tobias Bonhoeffer will introduce the essential role of neurotrophins, such as BDNF, in long-term potentiation and will present recent data on the receptor mechanisms involved. Volkmar Lessmann will continue by presenting work on the sites and mechanisms of neurotrophin secretion from neurons, a topic that has become a major focus in the field. Finally, Arthur Konnerth will show exciting new data on the mechanism of a fast, transmitter-like postsynaptic action of BDNF that appears to play an unexpected role in long-term potentiation.

In the second part of the symposium, Rüdiger Klein will focus on transsynaptic interaction of membrane-bound molecules and will present new work in the rapidly expanding field of the synaptic role of ephrins and eph receptors. This will be followed by a talk (Kay Jüngling/Kurt Gottmann) on the classical cell adhesion molecule N-cadherin regulating presynaptic function. Work employing in vitro differentiation of N-cadherin-deficient embryonic stem cells will be presented. Concluding the symposium, Frank Pfrieger will present the recent discovery, that cholesterol released by glial cells is an essential player in synapse formation and functional plasticity.

186 Activity dependent plasticity: Neurotrophins and morphological changes at the synaptic level.

Tobias Bonhoeffer

Max Planck Institute of Neurobiology, Germany

Hippocampal long-term potentiation (LTP) is generally thought to be induced by concurrent pre- and postsynaptic activity leading to elevated calcium levels in the postsynaptic cell. How the subsequent synaptic enhancement is achieved and later maintained and which molecules are important in this process is much less clear.

I will present evidence for brain-derived neurotrophic factor (BDNF) being one crucial important component in this system. BDNF-knockout animals show severely compromised LTP and long-lasting LTP is even completely abolished. It can be rescued by locally infecting cells with an adenovirus vector containing the BDNF gene, indicating that the BDNF protein is required at the time of LTP-induction. Moreover thetaburst-induced LTP is substantially diminished in the presence of a function-blocking BDNF-antibody. Interestingly the attenuation with the BDNF-antibody is as strong as with a trkB receptor-body which blocks both BDNF and NT4 action. This implies that BDNF but not NT4 is involved in hippocampal LTP.

Since it is well known that BDNF can influence the morphology of neurons, it is attractive to speculate that this molecule might provide a link between functional and morphological aspects of synaptic plasticity.

We and others have recently also been able to show that, indeed, there are morphological changes that occur when functional changes in synaptic strength occur. For some tens of year it had been difficult to prove this old idea, mainly because it has not been easy to pin-point the location of the synapses which are expected to change. We have now tackled this problem by combining two-photon imaging with a local superfusion technique thereby confining the region on the postsynaptic dendrite where the synaptic changes could occur.

We were able to show that local LTP induction in such a restricted region of the dendrite reliably led to the appearance of new spines in this area. These novel spines remained stable in shape and position for the whole period of observation, which lasted up to 24 hours. We found furthermore that the disappearance of spines was not, as is the case in LTP and spine growth, controlled in a specific and activity-dependent manner but it rather occurred more or less randomly in time and space.

The most attractive explanation for the formation of additional spines is a concurrent emergence of new synapses on these structures. Although more experiments on the precise nature of these changes are necessary, our data provide strong evidence that in the mammalian hippocampus not only physiological but also structural changes play an important role when neurons change the efficacy of their connections.

Synaptic targeting and secretion of neurotrophins 187

Volkmar Leßmann

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The protein family of the mammalian neurotrophins (consisting of NGF, BDNF, NT-3, and NT-4/5) is known to regulate the survival and the differentiation of PNS and CNS neurons. Recent evidence suggests that especially BDNF plays an additional important role in mediating activity-dependent synaptic plasticity. In this respect, BDNF has been shown to enhance within minutes glutamatergic synaptic transmission in hippocampal neurons by either facilitating glutamate release from presynaptic terminals (Lessmann and Heumann, 1998) or by enhancing postsynaptic NMDA receptor function (Levine et al., 1998). Furthermore, BDNF has been shown to be involved in LTP. Thus, BDNF is among the candidate molecules mediating activity-dependent synaptic plasticity at glutamatergic CNS synapses. However, direct demonstration of activity-dependent synaptic secretion of BDNF upon high frequency synaptic stimulation has long remained elusive.

Here we investigated secretion of BDNF labeled with green fluorescent protein (BDNF-GFP), by monitoring the changes in fluorescence intensity of BDNF-GFP secretory vesicles at glutamatergic synapses. Hippocampal neurons were transfected at 8 DIV with a BDNF-GFP fusion construct and BDNF-GFP secretion was monitored at 11-14 DIV. Using FM 4-64 labeling of active synapses, we selected for synaptic BDNF-GFP vesicle clusters in dendrites. The decrease in intradendritic fluorescence intensity (reflecting release of BDNF-GFP) was recorded by time lapse video microscopy. Stimulation with 50 mM K^+ resulted in a graded decrease of fluorescence intensity of BDNF-GFP vesicles in postsynaptic structures (τ= 300s ± 24s), yielding 59 ± 2% (n=17 cells) of the initial fluorescence intensity after 10 min of depolarization. Depending on the stimulation conditions, additional extrasynaptic secretion of BDNF-GFP was also evident.

Secretion of BDNF-GFP was critically dependent on the influx of extracellular Ca^{2+} and was abolished by preincubation with tetanus toxin (20 h, 1 nM) which blocks Ca^{2+} dependent vesicular release processes. High K^+ induced secretion of BDNF-GFP was blocked in the presence of 1 µm TTX, indicating the dependence of BDNF secretion on the generation of action potentials, whereas mere long-lasting depolarization was ineffective.

Importantly, synaptic release of BDNF-GFP upon high frequency electrical presynaptic stimulation (16x 1s bursts at 50 Hz) was strictly dependent on postsynaptic depolarization via ionotropic glutamate receptors, supporting the view that secretion of BDNF-GFP takes place from postsynaptic structures.

Direct intracellular depolarization of BDNF-GFP expressing neurons via patch clamp electrodes allowed to determine the required patterns of back-propagating action potentials in the dendritic tree that allow for dendritic secretion of BDNF.

Similar to BDNF, we constructed GFP fusion proteins of NGF, NT-3 and NT-4/5, respectively. The observed cellular fluorescence patterns suggest targeting of NT-3-GFP predominantly to secretory granules (similar to BDNF-GFP), whereas NGF and NT-4/5 seem to be targeted primarily to the constitutive pathway of secretion.

Taken together, these results provide direct evidence that BDNF is a possible synaptic messenger in activity-dependent plasticity of glutamatergic synapses. Future studies using the different GFP-tagged neurotrophins will enable to monitor secretion of all neurotrophins in intact synaptic networks.(Supported by grant SFB 553 TP C12 from the DFG)

Lessmann V, Heumann R . Neuroscience 86, 399-413. (1998)
Levine ES et al. PNAS. 95, 10235-39. (1998)

188 Regulation of glutamatergic transmission through BDNF-evoked dendritic depolarization

Arthur Konnerth

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Brain-derived neurotrophic factor (BDNF) and other neurotrophins are essential for the normal function of the mammalian nervous system. A rapid, transmitter-like neuroexcitatory action of BDNF is found in many types of central neurons. There is accumulating evidence that this action of BDNF plays important roles in neuronal (and glial!) signaling and in activity-dependent synaptic plasticity. By imaging dentate granule cells in mouse hippocampal slices, we found that pulse-like BDNF applications evoke Ca^{2+} transients, which are associated with the previously reported rapid depolarization (Kafitz et al., 1998). The BDNF-evoked response was reliably obtained in the cell's soma and in dendrites, but not in the axon. Particularly large Ca^{2+} signals were detected in dendritic spines. Pairing a weak burst of synaptic stimulation with a brief dendritic BDNF application caused an immediate and robust induction of long-term potentiation (LTP) (Kovalchuk et al., 2002). The LTP induction process involved activation of both postsynaptic Ca^{2+} channels and NMDA receptors. We conclude that spiny dendrites of mature dentate granule cells are highly responsive compartments for the rapid BDNF-action. The findings indicate a surprisingly fast and instructive role for BDNF in the induction of LTP. By screening candidate genes with an antisense mRNA expression approach and by co-expressing the receptor tyrosine kinase TrkB and various sodium channels, we found that the tetrodotoxin-insensitive sodium channel Nav1.9 underlies the neurotrophin-evoked excitation (Blum et al., 2002). These results clarify the molecular basis of neurotrophin-evoked depolarization and reveal a novel mechanism of ligand-mediated sodium channel activation.

1. Kafitz, K.W., Rose, C.R., Thoenen, H., Konnerth, A. (1999) Neurotrophin-evoked rapid excitation through TrkB receptors. Nature 401, 918-921.
2. Kovalchuk, Y., Hanse, E., Kafitz, K.W., Konnerth, A. (2002) Postsynaptic Induction of BDNF-Mediated Long-Term Potentiation. Science 295, 1729-1734.
3. Blum, R., Kafitz, K.W., Konnerth, A. (2002) Neurotrophin-evoked depolarization requires sodium channels $Na_v1.9$. Nature 419, 687-693.

Ephrins and Eph receptors in neuronal development and synaptic plasticity 189

Ruediger Klein

Max-Planck Institute of Neurobiology

Ephrins are membrane-bound ligands for Eph receptor tyrosine kinases (RTKs), the largest subfamily of RTKs in the mammalian genome. Because ephrins are non-soluble ligands, they regulate cell-to-cell mediated communication during a variety of developmental processes including cell migration, compartment boundary formation and segmentation, neuronal networking and angiogenesis. Recent work indicates roles for ephrins and Ephs during adulthood including neuronal plasticity. The B-subclass of ephrins consists of transmembrane proteins that display cell autonomous signaling potential upon engagement of Eph receptors on neighboring cells. This allows signals to propagate either into Eph-expressing cells (forward signaling), into ephrin-expressing cells (reverse signaling), or into both cells (bi-directional signaling). Recent advances in ephrin biology with focus on synaptic plasticity and their mechanisms of action will be reviewed.

Regulation of presynaptic function by synaptic adhesion molecules: Role of N-cadherin 190

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The synaptic cell adhesion molecule N-cadherin is found in the perisynaptic region of the pre- and postsynaptic membrane and mediates transsynaptic adhesion in a homophilic, Ca^{2+}-dependent manner. Therefore, N-cadherin has been proposed to play a major role in synaptogenesis. However, N-cadherin deficient mice die very early in embryonic development because of a failure of heart formation (1), thus impedeing the analysis of the nervous system.

To study effects of the N-cadherin gene knock-out on glutamatergic synapses, we differentiated mouse embryonic stem (ES) cells carrying the mutated N-cadherin gene *in vitro* to neurons and studied synaptic function. ES-cell derived neurons were purified by immunopanning, cultured in a microisland culture system and patch-clamp recordings were performed. Homozygous N-Cadherin deficient neurons (N-Cad -/-) showed a significantly decreased AMPA mEPSC frequency as compared to heterozygous N-Cad ± neurons and wild-type neurons. No effects on AMPA mEPSC amplitudes were observed. In paired-recordings, N-Cad -/- neurons showed an increase in synaptic latency and a decrease in charge flow during evoked AMPA EPSCs as a result of repeated stimulation (10sec interval). Most intriguingly, repeated stimulation led also to a strong desynchronization of vesicle release. In addition to the proposed role of N-cadherin in synapse formation, the complete absence of N-cadherin revealed unexpected effects on presynaptic transmitter release properties at glutamatergic synapses. In an use-dependent manner, transmitter release became strongly asynchronous suggesting a close link of N-cadherin function and the release process itself.

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191 Role of cholesterol in synapse development

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The mechanisms that mediate the formation and maturation of synapses in the mammalian central nervous system are still incompletely understood. A possible source of signals that control these fundamental processes are glial cells, namely astrocytes. This is indicated by the fact that in many brain areas, most synapses are formed after the differentiation of astrocytes. Previous studies on highly purified retinal ganglion cells have shown that glia-derived factors enhance synapse formation and maturation. In the first part of µ talk, I will summarize evidence that one of the synaptogenic factors is cholesterol (Mauch et al., 2001) and outline the hypothesis that massive synaptogenesis requires delivery of cholesterol by astrocytes via apolipoprotein E-rich lipoprotein particles. In the second part, I will present new results that address the question how cholesterol enhances synapse formation. In principle, cholesterol may 1) exert a signaling function, after conversion to a neurosteroid, 2) serve as building material for synaptic components like vesicles or 3) enhance the efficacy of cellular processes that mediate synapse assembly. To distinguish between these possibilities, we determine the effects of cholesterol derivatives, which cannot be converted to steroids or serve as raft components, on neuronal cultures study the time course of cholesterol-induced effects on synapses and analyse effects of cholesterol on processes like axonal transport that are essential for synapse development. Scrutinizing the dependence of synapse development on cholesterol may help to elucidate cellular processes that guide synapse assembly and support their stability.

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192 Regulation of glutamatergic transmission through BDNF-evoked dendritic depolarization

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Brain-derived neurotrophic factor (BDNF) and other neurotrophins are essential for the normal function of the mammalian nervous system. A rapid, transmitter-like neuroexcitatory action of BDNF is found in many types of central neurons. There is accumulating evidence that this action of BDNF plays important roles in neuronal (and glial!) signaling and in activity-dependent synaptic plasticity. By imaging dentate granule cells in mouse hippocampal slices, we found that pulse-like BDNF applications evoke Ca^{2+} transients, which are associated with the previously reported rapid depolarization (Kafitz et al., 1998). The BDNF-evoked response was reliably obtained in the cell's soma and in dendrites, but not in the axon. Particularly large Ca^{2+} signals were detected in dendritic

spines. Pairing a weak burst of synaptic stimulation with a brief dendritic BDNF application caused an immediate and robust induction of long-term potentiation (LTP) (Kovalchuk et al., 2002). The LTP induction process involved activation of both postsynaptic Ca^{2+} channels and NMDA receptors. We conclude that spiny dendrites of mature dentate granule cells are highly responsive compartments for the rapid BDNF-action. The findings indicate a surprisingly fast and instructive role for BDNF in the induction of LTP. By screening candidate genes with an antisense mRNA expression approach and by co-expressing the receptor tyrosine kinase TrkB and various sodium channels, we found that the tetrodotoxin-insensitive sodium channel Nav1.9 underlies the neurotrophin-evoked excitation (Blum et al., 2002). These results clarify the molecular basis of neurotrophin-evoked depolarization and reveal a novel mechanism of ligand-mediated sodium channel activation.

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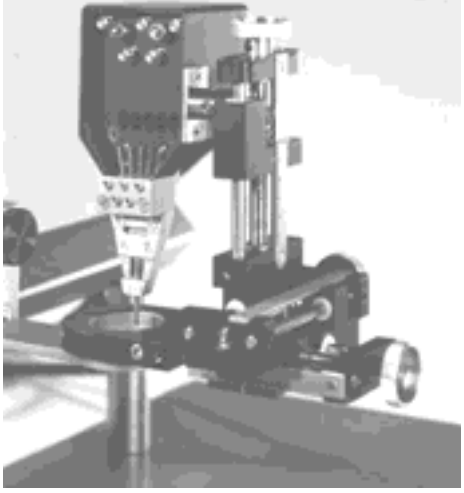

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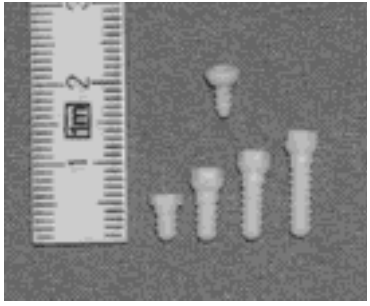

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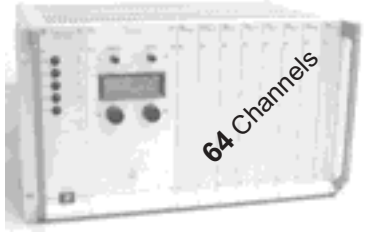

Introductory Remarks to Symposium 21

Molecular basis of axonal damage in inflammatory and degenerative CNS diseases

Harald Neumann and Mathias Bähr

Selective early loss of axons, dendrites and synapses is a common feature of several neurodegenerative and neuroinflammatory diseases including multiple sclerosis, traumatic or ischemic brain injury and Alzheimer's disease. Secondary degeneration of neurons is often the consequence of this primary neurite damage, resulting in permanent neurological deficits in patients.

The exact molecular mechanisms of primary axonal damage are not exactly known, but significant progress has been achieved in our understanding of the respective pathologies, as will be highlighted in the symposium:

In multiple sclerosis demyelination and inflammatory reactions are involved in axonal damage and loss of neurites (Brück and Nave). Axonal damage in multiple sclerosis is responsible for the permanent deficits of the patients. The damage of axons is mainly observed in the acute demyelinating multiple sclerosis lesions and is associated with the number of infiltrating macrophages and cytotoxic CD8+ T-lymphocytes.

In Alzheimer's disease intracellular polymerisation of axonal (mutated) proteins and extracellular amyloid aggregates appear to be the cause of axonal damage (Götz and Perry). Beta-amyloid plaques or polymerized beta-amyloid peptide can promote the formation of neurofibrillary tangles and loss of axonal teminals. Activation of the innate immune response (e.g. microglia, complement) is linked to the lesion site in Alzheimer's disease and might perpetuate the disease process.

Recently, experimental repair strategies have been designed to prevent further damage and functionally improve deficits caused by axonal and secondary neuronal degeneration: One exciting strategy aims at stimulating axonal outgrowth from lesioned neurons by interference with inhibitory signalling molecules such as Nogo (Kerschensteiner). Furthermore, stem cells that differentiate into oligodendrocytes are applied locally to induce remyelination (Brüstle).

In summary, the symposium will highlight the emerging research on the molecular mechanism of axonal damage and elucidate new ways for protective and cell replacement therapy.

193 Linking β-Amyloid Plaques to Neurofibrilary Tangle Formation in an Alzheimer's Disease Mouse Model

Jürgen Götz

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Tau pathology is a central neuropathological characteristic of a number of tauopathies including Alzheimer's disease (AD). In each disease the microtubule-associated protein tau is hyperphosphorylated and in a filamentous form. We reproduced the neurofibrillar pathology by transgenic expression of tau mutations associated with the inherited dementia FTDP-17. To test the amyloid cascade hypothesis which claims a central role of β-amyloid in neurofibrillary tangle formation, we injected β-amyloid into brains of tau transgenic and control mice. Beta-amyloid induced a fivefold increase in tangle formation in transgenic brains. The injections were followed by phosphorylation of tau at the phospho-epitopes Thr212/Ser214 and Ser422. A role of protein phosphatase PP2A in the dephosphorylation of tau and in the formation of neurofibrillary tangles is suggested by data we obtained in transgenic mice expressing a dominant negative mutant form of PP2A. Finally, to reproduce tau filament formation in tissue culture, we stably expressed human tau, with or without pathogenic mutations, in human neuroblastoma cells and exposed them to aggregated synthetic β-amyloid peptide. Both neuronally differentiated and undifferentiated cells developed numerous AD-like tau filaments. Together, our data support the amyloid cascade hypothesis. They help to map phospho-epitopes of tau linked to neurofibrillary tangle formation.

194 Inflammation in the CNS and its potential to trigger an axon "self destruct" programme

Hugh Perry

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It is now widely recognised that axon injury is a significant consequence of many types of injuries to, and diseases of, the CNS (Coleman and Perry, 2002). In multiple sclerosis T-cells and macrophages infiltrate the brain and secrete molecules which act as "molecular scissors" to transect axons. There is evidence to show that this T-cell/macrophage mediated injury is not necessarily immunologically specific and may involve a number of different inflammatory mediators including matrix metalloproteases and nitric oxide. The precise mechanisms by which these mediators produce axon injury is poorly understood. In the Wld mutant mouse axons undergo very slow Wallerian degeneration indicating that Wallerian degeneration in wild-type mice is an active process rather than a simple withering or passive degeneration process. Active degeneration of the cell body is typically associated with apoptosis but Wallerian degeneration has been shown to be a caspase independent form of degeneration. Molecular genetic studies have shown that the mutation in the Wld mouse involves a component of the ubiquitin-proteasome pathway. Understanding the role of this pathway in axon degeneration should lead to a better understanding of this important but relatively neglected aspect of neuropathology.

Early aspects of axonal damage in spinal cord injury 195

Martin Kerschensteiner

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How axons respond to transection is crucial for the outcome of spinal cord injuries. Three responses at the proximal axon stump are possible: neurite outgrowth, survival without growth or retrograde axonal degeneration. These processes can be distinguished morphologically and have been studied in fixed tissue. The dynamism of the responses of living axons, however, could only be inferred from these fixed specimen and thus far has only been studied either *in vitro* or in invertebrates.

The convergence of modern light microscopy and molecular biological techniques to label cells allows us for the first time to study the process of axonal transection in the CNS of living mice. As a first step we have performed time-lapse imaging of the early phase of traumatic axon damage for up to 4 hrs after experimental spinal cord injury. Our results indicate that early axon damage is a 2-step process consisting of temporally and morphologically distinct phases.

These studies can provide insight into the processes underlying an axon's response to transection and should thus lead to a better understanding of the molecular mechanisms underlying this process.

Axonal pathology in multiple sclerosis 196

Wolfgang Brück

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There is increasing evidence from morphological studies that axonal pathology such as axonal transections or acute axonal damage occurs in multiple sclerosis (MS) lesions. This is supported by recent magnetic resonance imaging (MRI) and magnetic resonance spectroscopic (MRS) studies which described hypointense lesions in T1-weighted MRI scans or decreases in the neuroaxonal metabolite N-acetylaspartate (NAA) in MS suggesting that axonal damage is responsible for the persistent neurological deficit in MS patients. The present talk will therefore cover the following topics: How extensive is axonal reduction in MS lesions?, is there a MRI or MRS correlate for axonal loss?, how can acute axonal damage be quantified and which immunopathological mechanisms are involved?

Axonal reduction in MS occurs early in plaque development as well as early in the course of the disease; there are, however, major variations in axonal reduction in individual MS patients. Axonal loss correlates with the degree of hypointensity in T1-weighted MRI, hypointensity is additionally affected by extracellular edema and the rate of demyelination or remyelination. Decreased NAA levels in MRS show a clear correlation with axonal reduction in MS lesions. Acute axonal damage can reliably be quantified by immunocytochemical detection of the Alzheimer precursor protein (APP). APP-positive structures were detected in all stages of demyelinating activity indicating that acute axonal damage probably occurs independent of active demyelination. The relevan-

ce of axonal loss for the development of clinical symptoms is investigated in cases of clinically silent MS. Different factors such as lesions site, axonal preservation or remyelination contribute to the clinical non-appearance of MS lesions.

197 Role of oligodendrocytes in axonal support and myelination

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In the mammalian brain, the best understood function of oligodendroglial cells is axonal ensheathment with the subsequent assembly of myelin. Throughout adult life, myelin sheaths remain in active metabolic contact to the oligodendrocyte soma, and can be considered as an "external" glial organell. Transgenic mice have shown that myelin plays an important role not only as an electric insulator, but also in axon-glia signaling and in long-term axonal suport. For example PLP/DM20, the major membrane protein of CNS myelin, is required for axonal function. We have shown that PLP-deficient mice develop normally, but their myelin is physically less stable, and mutants develop progressive signs of axonal degeneration. This has raised the question whether myelin instability and occasional delamination is possibly sufficient to cause secondary axonal involvement. We have now characterized mice with a mutation in Cnp1, a gene encoding 2',3'-cyclic nucleotide phosphodiesterase (CNPase) in oligodendrocytes. Surprisingly, also Cnp1 proves essential for axonal survival but not for the normal architecture of compact myelin. In the absence of glial CNP, mice develop axonal swellings and neurodegeneration throughout the brain, leading to hydrocephalus and premature death. Genetically, the function of glia in supporting axonal integrity has thus been completely uncoupled from maintaining compact myelin in these mice. We suggest that oligodendrocyte injury and cell death, such as in multiple sclerosis lesions, may suffice to cause secondary axonal loss.

Griffiths et al. (1998) Science 280, 1610-1613. Yool et al. (2001) J. Neurosci. Res. 63, 151-164.
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198 Stem cell-based therapy of demyelinating diseases

Oliver Brüstle

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Embryonic stem (ES) cells represent an unlimited donor source for neural repair. We have developed a variety of *in vitro* differentiation paradigms which permit the generation of ES cell-derived neurons and glia in high purity. Proliferative ES cell-derived oligodendrocyte progenitors can be obtained by a combination of controlled differentiation in growth factor-containing media and lineage selection with a construct carrying the bgeo gene under control of the CNP promoter. Efficient myelination is observed following transplantation of ES cell-derived glial precursors into the CNS of dysmyelinating mutants. Recent data suggest that this approach might also become useful for transplantation into inflammatory myelin disorders. Upon implantation into demyelina-

ted foci induced by the combined application of an anti-MOG antibody and complement, ES cell-derived glial precursors were found to survive and differentiate into oligodendrocytes which ensheathed host axons. The therapeutic application of ES cells in myelin repair will critically depend on the migration potential of the grafted cells. Overexpression of PSA-NCAM was explored as a strategy to enhance the migratory properties of ES cell-derived glial precursors. Retrovirus-mediated transfer of the gene encoding the PSA-synthesizing enzyme STX resulted in a six-fold increase in PSA-NCAM expression and enhanced the migratory potential of ES cell-derived glial precursors *in vitro*. In the long term, remyelination strategies might be supplemented by cell-mediated gene transfer. *In vitro* transplantation studies in slice cultures show that ES cell-derived astrocytes form gap junctions with the host glial network. This phenomenon could eventually be exploited for widespread therapeutic delivery of small molecules to the host CNS.

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Methyprednisolone increases neuronal apoptosis during chronic inflammatory disease of the CNS by inhibition of an endogenous neuroprotective pathway

199

Ricarda Diem [1], Muriel Hobom [1], Katharina Maier [1], Robert Weissert [2], Maria K. Storch [3], Roman Meyer [4] and Mathias Bähr [1]

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Optic neuritis is one of the most common clinical manifestations of multiple sclerosis (MS), a chronic inflammatory disease of the CNS(1). High-dosage methylprednisolone treatment has been established as the standard therapy of acute inflammation of the optic nerve (ON)(2). The rationale for corticosteroid treatment lies in the anti-inflammatory and immunosuppressive properties of these hormones as it has been shown in experimental autoimmune encephalomyelitis (EAE), the animal model of MS(3). However, steroids might not only have beneficial effects especially in the treatment of neurological disorders(4,5). To investigate the influence of methylprednisolone treatment on the survival of retinal ganglion cells (RGCs), the neurons that form the axons of the ON, we used a rat model of myelin oligodendrocyte glycoprotein (MOG)-induced EAE. Optic neuritis was diagnosed by recording visual evoked potentials, and RGC function was monitored by measuring electroretinograms. Methylprednisolone treatment over 3 days significantly increased RGC apoptosis during MOG-EAE. By western blot analysis, we identified the underlying molecular mechanism: a down-regulation of the phosphorylation of mitogen-activated kinases (MAPKs), an endogenous neuroprotective pathway. The methylprednisolone-induced inhibition of MAPK phosphorylation occurred in a calcium-dependent manner. Hence we provide evidence for negative effects of steroid treatment on neuronal survival during chronic inflammatory autoimmune disease of the CNS.

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200 Mechanisms and Time Course of Neuronal and Axonal Pathology in Experimental Autoimmune Encephalomyelitis

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Neuronal and axonal damage is considered to be the main cause for long-term disability in multiple sclerosis. We analyzed the mechanisms and kinetics of neuronal cell death in experimental autoimmune encephalomyelitis (EAE) induced by myelin oligodendrocyte glycoprotein (MOG) by combining an electrophysiological *in vivo* assessment of the optic pathway with the investigation of retinal ganglion cell (RGC) counts. In accordance with our previous findings in this animal model, neuritis of the optic nerve (ON) leads to apoptotic RGC death. By further investigating the time course of RGC apoptosis in the present study, we found that neuronal cell death together with decreased visual acuity values occurred before the onset of clinical symptoms. Kinetics of RGC death started at day 5 post immunization (π) and reached its maximum between day 7 π and day 1 of the clinically apparent disease. On day 8 of the disease, animals have lost more than 70 % of RGCs. Simultaneously with the time course of RGC apoptosis, we found a down-regulation of phospho-Akt as well as a shift in the relation of two proteins of the Bcl-2 family, Bax and Bcl-2, towards a more proapoptotic ratio in these cells. Comparing the kinetics and mechanisms of RGC death during MOG-EAE with those following complete surgical transection of the ON, we found significant agreement. Therefore, we hypothesize that axonal transection and functional impairment of the axons due to an inflammatory attack is the reason for neuronal cell loss in MOG-EAE.

Hypoxia-induced increase of intracellular calcium concentration in DRG neurons

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Hypoxic conditions observed at ischemia are the determinant of neuronal death during different forms of brain damage and pathology. The main mechanism triggering a damaging process is the excess glutamate release from synaptic terminals resulting in excessive increase of calcium ions in neurons and activation of different intracellular messenger systems leading to irreversible cell damage. This phenomenon of "excitotoxicity" has been extensively studied by numerous scientists. However, the intracellular mechanisms directly responsible for the damaging increase in intracellular calcium concentration are still not completely known. Therefore, the aim of our investigations was to study the mechanisms responsible for this phenomenon. In our experiments we used dorsal roots ganglia (DRG), which are suitable model for such kind of investigations. Intracellular calcium concentration was measured by spatial screening of isolated dorsal root ganglion neurons loaded with fluorescent dye Fura-2AM after subjecting them into moderate hypoxic solution (partial oxygen pressure=20 to 50mmHg). Short exposure of neurons to hypoxia resulted in a reversible elevation of intracellular calcium concentration to about 120% in the center and to 80% in the periphery of neurons. Such elevation could be almost completely (by 92%) eliminated by removal of calcium from external medium or application of L-type calcium channel blocker nifedipine. Replacement of external Na ions by Li ions or choline reduced the hypoxic effect less effectively (by 75%). Significant efficiency was achieved by preapplication of mitochondrial protonophore CCCP, which also decreased hypoxia-induced calcium elevation by 58%. The use of low cadmium concentrations to block plasmalemmal calcium ATPase did not alter the hypoxic effect. A conclusion is made that in isolated sensory neurons hypoxia-induced elevation of cytosolic calcium is induced by combined changes in three cell substructures: the functioning of voltage-operated L-type calcium and sodium channels as well as calcium accumulation by mitochondria. Hypoxia-induced changes in functioning of plasmalemmal sodium/calcium exchanger are of secondary role as a result of increased sodium influx into the cell. We concluded that mitochondria are important for spatial difference in the hypoxia-induced calcium elevation due to their specific location in these neurons.

202 Heat shock protein 70 (Hsp 70) expression in cerebellum in relation to ATP-ases activities in Morris hepatoma bearing rats.

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The cerebellar degeneration as a neurological paraneoplastic syndrome develops in course of lung, ovary and breast cancer. The pathomechanism taken in considerance is the autoimmune response of the host. Heat shock protein 70 is induced by such conditions like ischemia, seizures or hyperthermia. The aim of this paper was to study the expression of heat shock protein 70 in relation to ATP-ases activities in cerebellum in hepatoma Morris 5123 bearing rats. The tumor was inoculated intramuscullarly and after 21 days the animals were sacrified. The microscopical examination was performed in tumor for the confirmation of the activity of neoplasm, and in brain to exclude metastases as well as for morphological examination of cerebellum. We found the linear atrophy of Purkinje cells. The biochemical analyses based on Western – Blotting of Hsp 70 and kinetic estimation of Na^+/K^+, Ca^{2+} and Mg^{2+} - ATPases.

We found increased expression of Hsp 70 in cerebellum. The activity of Na^+/K^+ -ATP-ase was higher in Morris hepatoma bearing rats (264,3 ± 18,9 vs 71,6 ± 12,3 µmol Pi/min/mg protein in controls, $p<0.01$), as well as Ca^{2+} -ATP-ase (253,0 ± 30,2 vs 63,78 ± 16,4 µmol Pi/min/mg protein in controls, $p<0.01$). The decrease in activity of Mg^{2+} -ATPase was noticed in hepatoma rats (53,0 ± 16,0 vs 94,8 ± 32,5 µmol Pi/min/mg protein in controls, $p<0.05$). The increased expression of Hsp 70 followed by higher activities of Na^+/K^+ - and Ca^{2+} -ATP-ase seems to be one of pathomechanisms involved in subacute cerebellar degeneration.

Edvard Munch (1863-1944)

Introductory Remarks to Symposium 22

Neurotrauma: a trigger for schizophrenia?

Hannelore Ehrenreich, Anna-Leena Sirén and Eckart Rüther

Despite general agreement on the significance of a genetic predisposition, the etiology of schizophrenic psychosis remains obscure. There is, however, strong evidence for a number of co-factors (e. g. neurotrauma, drug abuse) that influence manifestation and course of schizophrenia. These findings point to a dual origin of the disease-determining processes: neurodevelopmental and neurodegenerative.

Epidemiological studies have established a connection between head injury and psychosis. As will be demonstrated by D. Malaspina, traumatic brain injury (TBI) significantly increases the risk for schizophrenia. Progressive deterioration of cognitive functions together with progressive ventricular enlargement in imaging studies are typical features of post TBI and schizophrenia supporting a neurodegenerative component in the pathophysiology of schizophrenia. The possible mechanisms underlying this connection will be further addressed in the presentation of A.-L. Sirén and H. Ehrenreich demonstrating distinct and selective behavioral deficits and significant enlargement of the ventricle system months after application of a discrete lesion of the right parietal cortex in mice. The presentation of J. Price will address the role of possible reactivation of developmental gene expression after brain damage. His contribution will provide evidence for a role of OCT-6 as a pathophysiological marker that is turned on in both schizophrenia and after neurotrauma. Using high-resolution MRI scans, Giedd will provide evidence for continuous degenerative processes in schizophrenia. His studies demonstrate dynamic patterns of accelerated gray matter loss in the brains of childhood-onset schizophrenia with earliest defects in the parietal association cortex, an area where gray matter loss is known to be strongly associated with environmental risk factors such as TBI. A reduction in interneuronal neuropil in the prefrontal cortex that has been shown to be a prominent feature of cortical pathology in schizophrenia suggesting that subtle changes in cellular architecture and brain circuitry may have a devastating impact on cortical function. T. Falkai will address the role of inflammatory mechanisms in these events. T. Pollmächer will further elaborate on involvement of cytokines in the pathophysiology of schizophrenia.

203 Neurotrauma and Schizophrenia: Epidemiology

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Neurotrauma and later serious psychopathology have long been associated. Kraepelin, in 1919, stated that head injuries in childhood might either cause or release predisposition to praecox, implicating a causative role for neurotrauma in the risk for psychosis. Indeed, neurotrauma is a reasonable exposure to examine with respect to later schizophrenia, as it is common and it has been associated with a range of neuropsychiatric conditions, including depression, anxiety, cognitive dysfunction, aggressive disorders and Alzheimer's disease. Neurotrauma could cause a phenocopy of genetic schizophrenia or interact with schizophrenia vulnerability genes to increase schizophrenia risk. However the association of neurotrauma and schizophrenia does not prove that it is a causative factor. It could also be spuriously associated with schizophrenia if those who are predisposed to develop schizophrenia just have greater amounts of trauma for other reasons, such as motor or attentional deficits. We approached this question by examining neurotrauma and diagnosis in the members of multiplex bipolar and schizophrenia pedigrees. Our data showed that members of schizophrenia pedigrees had greater rates of neurotrauma than members of bipolar pedigrees, even in relatives without schizophrenia. Neurotrauma did not increase risks for psychosis or schizophrenia in bipolar pedigrees members, but it doubled the risk of a schizophrenia diagnosis in those with genetic schizophrenia vulnerability.

In conclusion, the increased schizophrenia that accompanies a history of neurotrauma may result from an interaction of genetic schizophrenia vulnerability with the molecular cascade that results from neurotrauma exposure.

204 Late consequences of neurotrauma

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The molecular mechanisms leading to late consequences of neurotrauma and posttraumatic stress disorder are largely unclear. Clinical studies have established a causal relationship between traumatic brain injury (TBI) or psychotrauma and psychoses. Also, the causal relationship between TBI and Alzheimer disease (AD) has been recognized in retrospective clinical studies. These pathologies are associated with decreased cognitive function, significant disability and disturbed quality of life. Prospective human studies are missing, however, and our knowledge of the functional, morphological and molecular consequences of experimental neurotrauma is limited to observations with a short follow-up of a few weeks. Thus, relevant animal models to be used in long-term follow-up setting are of pivotal importance for advancement of knowledge in this field.

We have established a neurotrauma model in rodents (mice/rats) that allows long-term functional and morphological follow-up. We have shown that a small, standardized unilateral parietal cortical trauma in rats and mice results in long-lasting bilateral hippocampal alterations in gene and protein expression, in abnormal behavior, and in bilateral

hippocampal cell loss. Our previous data on the hematopoietic growth factor, erythropoietin (EPO) has revealed that EPO can act neuroprotective both in animals and man via multiple mechanisms of action during neurodevelopment and metabolic stress. EPO treatment in the mouse model of neurotrauma improved functional and morphological recovery after neurotrauma. Based on these findings we have formulated the following working hypothesis: Neurotrauma, in particular in the presence of a genetic predisposition, can act as an inducer / enhancer of neurodegeneration via activation of cellular signaling cascades that conjure to a final common deleterious pathway. Our goal is to characterize the genes of this "final common pathway", to understand the interplay between neurotrauma and genetic predisposition in activation of this cascade and to test the characterized novel strategies for therapeutic neuroprotective interventions.

Oct-6, Neural Damage, and Schizophrenia

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Oct-6 is a POU-III domain transcription factor, whose only known role in the nervous system is developmental. In the rodent, it is expressed in pre-myelinating Schwann cells, and in the brain by young migrating telencephalic neurons. Postnatally, expression is retained by a subset of pyramidal neurons in cortical layers 3 and 5 and in the hippocampal CA1 field, before total expression is lost at about 30 weeks of age. Although normal adult brain does not express Oct-6, we have discovered that Oct-6 is expressed in two neuropathological situations. First, it is expressed by neurons following ischaemic or neurotoxic damage. Interestingly, such expression is very long-lived being detectable many months after damage first occurred. Moreover, expression is only turned off if the brain is repaired by transplantation of neural stem cells, which bring about functional and structural recovery following these types of damage. The second scenario is that Oct-6 is expressed in a subset of neurons in post-mortem schizophrenic tissue in both temporal and frontal lobes. This expression appears not to correlate with neuroleptic exposure. One hypothesis to explain this finding is that a brain insult during the period of normal developmental Oct-6 expression leads to a subsequent failure to turn off the gene. We tested this hypothesis experimentally by lesioning newborn mice cryogenically, then examining Oct-6 expression when the animals matured. We discovered that cryogenic injury during the period of normal Oct-6 expression prevents its subsequent down-regulation in adulthood. This is consistent with the hypothesis that developmental damage can lead to the Oct-6-expressing phenotype that we observe in schizophrenia patients.

206 Cortical gray-matter deficits in schizophrenia

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Neurodevelopmental models for the pathology of schizophrenia propose both polygenetic and environmental risks, as well as early (pre/perinatal) and late (usually adolescent) developmental brain abnormalities. Using novel brain mapping algorithms, we detected striking anatomical profiles of accelerated gray matter loss in very early-onset schizophrenia; deficits moved in a dynamic pattern enveloping increasing amounts of cortex throughout adolescence. Early-onset patients were re-scanned prospectively with MRI, at two-year intervals at three time-points, to uncover the dynamics and timing of disease progression during adolescence. The earliest deficits were found in parietal brain regions, supporting visuo-spatial and associative thinking, where adult deficits are known to be mediated by environmental factors. Over 5 years, these deficits progressed anteriorly into temporal lobes, engulfing sensorimotor and dorsolateral pre-frontal cortices, and frontal eye fields. These emerging patterns correlated with psychotic symptom severity, and mirrored the neuromotor, auditory, visual search and frontal executive impairments in the disease. In temporal regions, gray matter loss was completely absent early in the disease but became pervasive later. Only the latest changes included dorsolateral prefrontal cortex and superior temporal gyri, deficit regions found consistently in adult studies. These emerging dynamic patterns were (1) controlled for medication and IQ effects, (2) replicated in independent groups of males and females, and (3) charted in individuals and groups. The resulting mapping strategy reveals a shifting pattern of tissue loss in schizophrenia. Novel aspects of the anatomy and dynamics of disease are uncovered, in a changing profile that implicates genetic and non-genetic patterns of deficits

207 The neuropathological basis pointing at a progressive illness in schizophrenia

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There is an ongoing debate whether schizophrenia is a neurodevelopmental disorder or a progressive illness. Based on neuropathological studies there are the following lines of evidence:

1. Changes: Lack of a significant degree of cell loss and rather subtle cytoarchitectonic changes point at neurodevelopment arrest e.g. in the second trimester of gestation.

2. Macrogliosis /Microgliosis: A typical degenerative brain process is characterised by cell loss and increased density of macroglia (astrocytosis). There is no evidence for macrogliosis/astrogliosis in schizophrenia. Whether there is increase of activated microgliosis is a matter of ongoing research; there is about the same number of positive and negative findings.

3. Progressive Changes: Imaging studies suggest progression of ventricular enlargement and cortical atrophy over time. Progressive changes without degeneration seems possible on the basis of our recent knowledge on neuronal plasticity and neuronal integrity.

In summary the neuropathological changes seen in schizophrenia seem to support a neurodevelopmental basis combined with an additional progressive component over the course of the illness.

The involvement of cytokines in the pathophysiology of schizophrenia

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Experimental findings in animals and humans suggest that inflammatory cytokines can induce acute detoriations of cognition, memory and mood. Moreover, cytokines are crucially involved in brain developed and in the survival of neurons. Hence, it is reasonable that these immune mediators might be involved in the pathogenesis and pathophysiology of schizophrenia and that cytokine networks may represent interesting novel therapeutic targets. However, so far, genetic studies as well as investigations on cytokine networks in the brain and the cerebrospinal fluid of patients with schizophrenia did not produce convincing evidence for alterations of cytokine networks within the central nervous system (CNS). This negative evidence, though, is based on a small number of studies. In contrast, there are quite robust findings obtained from peripheral blood suggesting that IL-2, IL-6, TNF-a and IFN cytokine systems are regulated differently in schizophrenic patients as compared to controls. However, these findings are not specific to schizophrenia, their pathophysiological relevance for processes within the CNS is undetermined, and the respective results are confounded by numerous intervening variables such as stress, smoking and medication. Medication in particular has a profound influence on cytokine networks which might not only be important as a confounding factor but also for the understanding of the drugs‘ beneficial actions on the CNS. Therefore, further experimental research on the involvement of cytokines in the pathogenesis, pathophysiology and treatment of schizophrenia is urgently needed.

Introductory Remarks to Symposium 23

German-Israeli cooperation in neuroscience

Bert Sakmann, Heinz Beck and Marlies Dorlöchter

There is a long tradition of cooperation between German and Israeli scientists. The German Max-Planck-Society and the Israeli Weizmann Institute were the first to initiate official contacts between scientists from Israel and Germany in 1959, long before diplomatic relations between both countries were possible. Following these early contacts, the German Federal Ministry of Education and Research (BMBF) and the Israeli Ministry of Science, Culture and Sport (MOS) established a cooperation in various fields of medical research. Molecular and cellular mechanisms of brain function and neurological diseases are the main research areas of the current projects.

Using a combination of electrophysiological, molecular biological and optical imaging methods in addition to behavioral studies, A. Grinvald and B. Sakmann examine the functional architecture of the mammalian cortex. They address the development of cortical maps, their function and dysfunction with regard to glutamate receptor channels and other neuronal messenger systems.

H. Bergmann and A. Engel investigate the pathophysiological relevance of synchrony and temporal patterning in Parkinson's disease in animal models. Abnormalities in these characteristics of neuronal assemblies are critical for both the tremor and the negative motor signs of the disease such as akinesia and rigidity. Recording of basal ganglia and cortical activity in monkey and rat allows to study effects of pharmacological manipulations of the symptoms.

Y. Yaari and H. Beck examine activity-dependent molecular and functional changes in different classes of voltage-dependent ion channels, and how these changes affect the intrinsic firing behavior of neurons. Altered expression of voltage-dependent Na^+ and Ca^{2+} channels following epileptic activity was shown to cause a dramatic and long-lasting switch from regular to burst firing mode. This change may be critical for initiation of seizures and suggest new drug targets in the treatment of epilepsy.

Irreversible injury to neuronal structures in patients with multiple sclerosis (MS) is the topic of F. Zipp's and S. Brocke's work. They present evidence that tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) plays a detrimental role for neuronal injury in autoimmune encephalomyelitis, an animal model of MS. Functional blockade of the death pathway may open up a new therapeutic avenue for MS patients.

Nerve growth factor and the related neurotrophins are secreted by target cells in the projection fields of responsive neurons, which are dependent on this trophic support for survival and maintenance of phenotype. The cellular mechanism by which the neurotrophin signal is propagated from the axon terminal to the cell body is probably retrograde axonal transport of activated neurotrophin-receptor complexes. Work by M. Fainzilber and T. Jovin supports this „signaling endosome" hypothesis.

GABA, the main neurotransmitter in the suprachiasmatic nucleus (SCN) has a dual effect on SCN neurons, excitatory during the day, and inhibitory at night. This has been attributed to circadian changes in intracellular chloride concentration ($[Cl^-]i$). Indeed, Y. Yarom and F. Nürnberger demonstrate that GABA induced current in SCN neurons can significantly alter $[Cl^-]i$. Slow recovery from Cl^- depletion along with a reduced activity of the GABA transporter, GAT-1, can explain a lower $[Cl^-]i$ during the night phase of the circadian cycle.

209 Imaging Spatio-Temporal Dynamics of Surround Inhibition in the Barrels Somatosensory Cortex

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Sensory processing and its perception require that local information would also be available globally. Indeed, in the mammalian neocortex, local excitation spreads over large distances via the long-range horizontal connections in layer 2/3 and may spread over an entire cortical area if excitatory polysynaptic pathways are also activated. Therefore, a balance between local excitation and surround inhibition is required. Here we explored the spatio-temporal aspects of cortical depolarization and hyperpolarization of urethane-anesthetized rats. New voltage sensitive dyes were used for high-resolution real time visualization of the cortical responses to whisker deflections and cutaneous stimulations of the whisker pad. These advances facilitated imaging of ongoing activity and evoked responses even without signal averaging. We found that motion of a single whisker evoked a cortical response exhibiting one or three phases. During a tri-phasic response, there was first a cortical depolarization in a small cortical region having a size of a single cortical barrel. Subsequently, this depolarization increased and spread laterally in an oval-like fashion, preferentially along the barrel's rows. During the second phase, the amplitude of the evoked response declined rapidly, presumably due to recurrent inhibition. Subsequently, the third phase exhibiting a depolarization rebound was observed and clear ~16 Hz oscillations were detected. Stimulus conditions revealing a net surround hyperpolarization during the second phase were also found. The present findings shed light on the intricate spatio-temporal interplay between excitatory and inhibitory synaptic interactions in the neocortex.

210 Role of neural dynamics in Parkinson's disease – comparative physiological studies in the primate basal ganglia

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Adaptive behavior requires evaluation of environmental stimuli with respect to their behavioral significance. Dopamine (DA) and acetylcholine (ACh), acting in the striatum (the input stage of the basal ganglia), have been shown to emit signals that can aid such an evaluation. We recorded from both types of neurons and from neurons in the output structures of the basal ganglia (globus pallidus (GP) and substantia nigra reticulata (SNr)) in monkeys performing a probabilistic delayed response task, before and after induction of severe Parkinsonism by systemic MPTP treatment. We show that while the dopaminergic response reflects the mismatch between expectation and outcome, cholinergic neurons respond in the same manner to all behaviorally significant events. The percentage of correlated GP pairs significantly increase following MPTP treatment and is restored to nearly normal values under dopamine replacement therapy. We conclude that the normal basal ganglia activity represents an optimally compressed (uncorrelated) version of distinctive features (as selected by the complement messages of the DA and ACh signals) of cortical information. Changes in basal ganglia synchronization are

caused by DA depletion and are correlated with the clinical manifestations of Parkinsonism and its pharmacological treatment.

Plasticity of Intrinsic Neuronal Excitability in Hippocampal Principal Neurons Following Status Epilepticus 211

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A single episode of status epilepticus (SE), induced by the convulsant pilocarpine, leads to the development of a chronic epileptic condition by causing long-lasting changes in synaptic and intrinsic neuronal properties. One of the most dramatic changes is the appearance of intrinsic bursting in nonbursting neurons. Thus, ~80% of CA1 pyramidal cells, which normally fire in a regular mode, are persistently converted to a bursting mode. A similar transformation, albeit less remarkable, occurs in CA3 pyramidal cells, but not in dentate granule cells. Pharmacological analysis in CA1 pyramidal cells suggests that persistent Na^+ current (INaP) contributes to somatic bursting behavior in most SE-experienced neurons. Additionally, a dendritic T-type Ca^{2+} current (ICaT) that is recruited by back-propagating action potentials, reinforces somatic bursting in many neurons. In accordance with these data, we find that INaP, and more dramatically ICaT, are persistently upregulated following SE. We suggest that upregulation of these two currents in hippocampal pyramidal cells contributes to the predominance of intrinsic bursting patterns after SE, and hence, to the evolution of epileptogenic foci in the hippocampus.

Regulation of neuronal apoptotic cell death in autoimmune inflammatory disorders of the central nervous system 212

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In patients with multiple sclerosis (MS), long-term neurological disability correlates with irreversible injury to neuronal structures rather than with the degree of demyelination. Thus, identification and prevention of the underlying damage mechanisms constitute an important therapeutic issue. The goal of this German-Israel project was to investigate and modulate both pro- and antiapoptotic mechanisms of neuronal injury induced by myelin-specific T cells which invade the brain during MS and the MS model disease experimental autoimmune encephalomyelitis (EAE).

The first data set provides conclusive evidence for a detrimental role of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) for neuronal injury in EAE. Clinical severity was substantially ameliorated by blockade of this death ligand, and treated animals showed a reduced number of apoptotic neurons. Conversely, the application of the ligand led to a deterioration of the motor disability. The use of living organotypic

brain slices allowed us to analyze this effect, as neuronal damage induced by encephalitogenic lymphocytes was decreased upon inhibition of TRAIL, following incubation wiht TRAIL -/- T cells, T cells recognizing a non-brain antigen, and allogenic T cells.

Since TRAIL is upregulated on antigen-specific T cells and induces neuronal death in living human brain slices, the presented data show that functional blockade of this death pathway may open up a new therapeutic avenue for multiple sclerosis patients.

213 Retrograde signaling in healthy and injured neurons

Francisca Bronfman, Marianna Tcherpakov, Shlomit Hanz, Eran Perlson, Tom Jovin and Mike Fainzilber

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A combination of cell biology, proteomics and fluorescence methods have been used to identify and characterize retrograde signaling complexes in neurons, ranging from signaling endosomes of the p75 neurotrophin receptor, to nuclear-targeted complexes formed in axoplasm after injury. We will present the latest update of our results on these research lines.

214 Gaba, Chloride and Circadian Rhythm.

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There is hardly a facet of mammalian physiology and behavior that is not subject to diurnal variation and control, from sleep/wakefulness and endocrine function to thermoregulation and metabolism. The best-defined master oscillator that orchestrates these myriad functions is the brain's circadian clock, which resides in the hypothalamic suprachiasmatic nucleus (SCN). We have previously shown that GABA, the main neurotransmitter in the SCN, has a dual effect, excitatory during the day, and inhibitory at night. This duality has been attributed to changes in intracellular chloride concentration ($[Cl^-]_i$) along the circadian cycle. Using voltage-clamp methodology in conjunction with local application of GABA, we demonstrated that GABA induced current in SCN neurons can significantly alter $[Cl^-]_i$. The recovery of intracellular Cl^- was described by an exponential curve. Recovery following Cl^- depletion was slower than recovery from Cl^- loading, and was further reduced during the subjective night. Since GAT1, a GABA transporter, translocates chloride and sodium together with GABA, and since in the SCN this transporter is located on axon terminmals and glia, as well as on some dendrites, we studied the effect of the GAT1 inhibitor SKF-89976A on the recovery of the intracellular chloride concentration. While recovery from chloride loading was nearly unaffected by the blocker, recovery from chloride depletion was reduced, particularly during subjective day .We concluded that a) SCN neurons express a large number of somatic GABAA receptors, which give rise to a modifiable, tonic Cl^- conductance that modulates cell excitability. b) Two Cl^- transport mechanisms operate in SCN neurons: One that replenishes the cell with Cl^- following Cl^- depletion, and another that removes Cl^- after

Cl^- loading. c) The reduction in efficiency of the replenishing mechanism during the subjective night is most likely due to the reduction in the activity of GAT-1 transposter. d) This reduction can explain a lower $[Cl^-]_i$ during the night phase of the circadian cycle.

Encephalitogenic T cells induce neuronal cell death in autoimmune encephalomyelitis via TRAIL 215

Orhan Aktas [1], Stefan Brocke [2], Alina Smorodchenko [1], Carmen Infante-Duarte [1], Timour Prozorovski [1], Venera Osmanova [2], Erik Kwidzinski [3], Elena Pohl [3], Martin Beyer [1], Ingo Bechmann [3], Robert Nitsch [3] and Frauke Zipp [1]

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Only recently, it became clear that neurological disability in multiple sclerosis (MS) correlates with irreversible injury of neuronal structures rather than with demyelination. Here we provide conclusive evidence for a detrimental role of tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), a member of the TNF/NGF family, in T cell-mediated neuronal injury during murine adoptive transfer encephalomyelitis, an animal model of MS. Clinical deficits and number of apoptotic neurons were substantially prevented by brain-specific blockade of TRAIL prior to disease manifestation. Using living brain tissue, we found that neuronal damage induced by encephalitogenic lymphocytes was decreased upon inhibition or knockout of TRAIL. Thus, the selective blockade of TRAIL-mediated neurotoxic T cell effects during neuroinflammation may open up a new therapeutic avenue for MS patients.

Diurnal reactivity patterns of glutamic-acid decarboxylase in the suprachiasmatic nucleus of the golden hamster 216

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GABA is the most prominent transmitter within the suprachiasmatic nucleus (SCN), which represents the central nervous circadian clock; more than 80 % of the neurons of the SCN use this messenger. The key enzymes for the synthesis of GABA is glutamic-acid decarboxylase (GAD) which occurs in two major isoforms: the 65kDa isoform (GAD-65) and the 67kDa isoform (GAD-67). Since distinct differences were described for the synthetic abilities of these two isoforms, the analysis of the relative involvement of GAD-65 or GAD-67, respectively, in the decarboxylation of glutamic acid is important for understanding the synthesis process of GABA in neurons of the SCN. Accordingly, we studied the principal occurrence of GAD-65 and GAD-67 in the SCN of golden hamsters, a species often used for biorhythm research, and compared the DNA sequence with that of rat and mouse. Further, we studied the content of both isoforms duirng different diurnal phases, and, by use of hamster-specific mRNA probes, we analyzed their expression levels.By the use of immunocytochemistry, the GAD isoforms

were observed in virtually all neurons of the SCN. The sequence analysis of the GAD-65-cDNA fragment of the hamster showed a 95.0 % homology with that of the rat and a 94.5 % homology with that of the mouse, whereas the homology of GAD-67 was 94.5 % and 94.3 %, respectively. In-situ hybridization revealed the highest level of GAD-65 mRNA at the beginning of the dark phase. The minimum was observed at the beginning of the light phase. GAD-67 did not show significant fluctuations during the day night cycle. As analyzed by immunoblotting, GAD-65 proved to be most abundant during midnight, thus, with a conspicuous phase delay to the expression maximum. This finding is of striking interest, since the highest levels in the content of GABA were found during the late night. This reactivity pattern of GAD represents a straight synthetic pathway from the processing of the GAD-65 enzyme to the availability of the transmitter GABA. GAD-67 does not seem to be involved in the alterations of the suprachiasmatic GABA synthesis.

217 The suprachiasmatic GABA neuron: Relation of input and output factors with the day-night cycle

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The suprachiasmatic nucleus (SCN) is the main circadian oscillator within the mammalian brain. The circadian rhythm is generated by a multi-oscillator network formed by the individual neurons of the SCN, which are reported to represent basal oscillators ("clock cells") expressing diverse phase stages. It is suggested that γ-amino butyric acid (GABA) plays a key role in the generation of the general circadian rhythm and its synchronization with the exogenous 24 h rhythm. In the present study, various components of the GABAergic system including the glutamatergic input systems from the retina transmitting the ambient illumination situation and the GABAergic efferent systems were analyzed in golden hamsters during different phases of the day-night cycle. Semiquantitative analysis by the use of HPLC, immunocytochemistry and immunoblot technique resulted in significant diurnal fluctuations of all compounds studied. During the dark phase, the content of GABA and the reuptake of GABA from the synaptic cleft as verified by the expression of GABA transporters was highest. This was further substantiated by the drastic increase of nocturnal release of GABA from SCN-slice cultures. On the input side of glutamatergic retinohypothalamic projections, the maximum of the reactivity of the AMPA-receptor subunits GluR-1 and GluR-2/3 were observed during the light phase. On the output side, the subunits α 2, α 3, β 1 and β 2/3 of GABA-A receptor were verified. The upregulation of the GABAergic system during the night phase helps to synchronize the suprachiasmatic "clock cells". The synchronization of the GABA release with the day-night cycle is mediated by the glutamatergic system.

CNS Recruitment of Pathogenic T Lymphocytes by CXCL12 expressed at the apical brain endothelium 218

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Inflammatory demyelinating diseases are characterized by migration of pathogenic lymphocytes from blood into the brain and spinal cord, possibly due to autoimmunity. We demonstrate here that pertussis toxin (PTX), a blocker of chemokine-triggered Gi-protein mediated responses inhibits the accumulation of myelin antigen-specific T cells in the brain and prevents experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis (MS). We found that normal brain vascular endothelium constitutively expresses the chemokine CXCL12 (stromal cell-derived factor-1, SDF-1). CXCL12 significantly strengthens integrin-dependent shear-resistant adhesion of pathogenic T cell clones to VCAM-1, a major vascular adhesion receptor implicated in T cell recruitment into the central nervous system (CNS). CXCL12 also chemoattracts encephalitogenic T cells *in vitro*. AMD3100, a specific and potent inhibitor of the CXCL12 receptor CXCR4 blocks CXCL12-mediated T cell recruitment and reduces clinical and histopathologic signs of EAE. This is one of the first demonstrations that the CNS vasculature constitutively expresses a functional proadhesive and promigratory chemokine. CXCL12/CXCR4 interactions thus appear to regulate early T cell recruitment to the brain in demyelinating diseases, and may offer a potential novel target in blocking initial lesion formation during the development of MS.

Differential Regulation of VLA-4 on Encephalitogenic CD4+ and CD8+ T cells by the lymphoid chemokines ELC (CCL19) and SLC (CCL21) 219

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Autoimmune inflammatory diseases of the central nervous system (CNS) such as multiple sclerosis (MS) are possibly initiated by antigen-specific CD4+ or CD8+ T cells that are recruited to the CNS. These brain-specific T cells may cross the blood-brain barrier (BBB) and home to the CNS where they mediate inflammatory lesion formation and axonal damage.

Chemokines have been described to play a role in the recruitment of antigen-specific T cells to the brain. Furthermore, the expression of adhesion molecules, such as vascular cell adhesion molecule–1 (VCAM-1) and ICAM-1 at the BBB accelerates the trafficking of activated T cells to the brain. A central and critical question concerning the passage

and trafficking of activated CD4+ or CD8+ T cells to the brain relates to the molecules involved in the sequence of events that leads to the full development of inflammatory lesions. We found that the lymphatic chemokine CCL19 is highly expressed on the BBB endothelium of mice challenged with experimental autoimmune encephalomyelitis (EAE), an animal model of MS. Furthermore, the lymphatic chemokine CCL21, which is undetectable in healthy CNS, is strongly expressed in brains of mice with EAE early in the course of the disease, and its expression proceeds the expression of prototypic pro-inflammatory chemokines such as IP-10, RANTES or MCP-1. The expression of chemokines at the BBB may trigger the activation of VLA-4 expressed on encephalitogenic T cells. In a flow chamber set up, we could show that CD4+ encephalitogenic T cells interact with immobilized vascular VCAM-1 in the absence of chemokines, whereas CD8+ T cells weakly interact with this vascular adhesion molecule. However, in the presence of CCL19 or CCL21, CD8+ T cells are strongly induced to tether and roll and adhere to VCAM-1/CCL19/21 in a VLA-4 dependent manner. In contrast, the presence of the chemokines only slightly increased the number of CD4+ T cells interacting with VCAM-1 under flow. Furthermore, in an transendothelial migration set up using the flow chamber, we found that the transendothelial migration of encephalitogenic CD4+ and CD8+ T cells was entirely dependent on the presence of chemokines such as CCL19, CCL21 and CXCL12, whereas in the absence of those chemokines no transendothelial migration or locomotion over the endothelium occured.

Our findings suggest a novel role for the chemokine-dependent differential trafficking of CD4+ and CD8+ encephalitogenic T cells to the brain. The fine regulation of chemokine receptors expressed by activated CD4+ or CD8+ T cells and chemokines, expressed at the BBB at different stages of the diseases can regulate the sequential influx of antigen-specific cells to the brain by activating leukocyte expressed integrins. The rapid activation of VLA-4 expressed on CD8+ T cells by immobilized CCL21 and the subsequent VLA-4-mediated arrest to VCAM-1 could allow the interaction with BBB-expressed VCAM-1 and trigger the early influx of CD8+ T cells into the CNS. This chemokine-dependent trafficking of CD8+ T cells to the CNS could lead to an early and transient accumulation of CD8+ T cells and a later recruitment and massive infiltration of CD4+ T cells, resulting in a fulminant inflammation of the CNS.

220 P2 receptors in satellite glial cells in trigeminal ganglia of mice

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There is ample evidence that signaling via nucleotide (P2) receptors is an important mode of communication in the nervous system. Neurons in sensory ganglia have been shown to express functional P2 receptors, which might play a role in the transmission of

pain signals. In contrast, virtually nothing is known about P2 receptors in satellite glial cells (SGCs), which are the main glial cells in these ganglia. We investigated the possibility that P2 receptors exist in SGCs in murine trigeminal ganglia, using Ca^{2+} imaging, patch-clamp recordings and immunohistochemistry. We found that ATP caused an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in SGCs. As adenosine had no effect on $[Ca^{2+}]_i$, and the P2 receptor antagonist pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS) largely blocked the response to ATP we conclude that P1 receptors did not contribute to the responses. The responses to ATP are likely to be mediated by P2Y receptors, because of (i) the persistence of the responses in Ca^{2+}-free solution, (ii) the inhibition of the response by cyclopiazonic acid, (iii) the large $[Ca^{2+}]_i$ increases in response to the P2Y agonists UTP, ADPbS, and 2MeSADP, and (iv) the failure of the P2X agonist a,bMeATP to elicit a response. Agonists of $P2Y_1$ receptors and UTP, an agonist at P2Y2 and P2Y4 receptors, induced $[Ca^{2+}]_i$ increases. Moreover, the SGCs were immunopositive for $P2Y_4$ receptors. Although $P2Y_1$ and P2Y4 receptors appear to exist on SGCs, additional P2Y receptor subtypes might also be present. Patch-clamp recordings of SGCs did not reveal any inward current due to ATP. Therefore, there was no evidence for the activation of ionotropic P2X receptors under the present conditions.

Novel adenosine 5'-O-(1-boranotriphosphate) derivatives induce subtype specific internalization of P2Y receptors

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P2Y-nucleotide receptors belong to the family of G-protein-coupled receptors. They are involved in numerous physiological and pathophysiological functions in the nervous system, thus representing important targets for drug development. The P2Y1-receptor (P2Y1R) is widely distributed in the different brain regions and is stimulated specifically by 2-MeSATP and ATP, whereas the P2Y2-receptor (P2Y2R), which is the only subtype that is equipotently stimulated by UTP and ATP, is detectable in the brain at lower levels (1). The lack of stable and receptor specific agonists however hampers studies for potential clinical applications of these receptors.

As recently reported by us (2), a novel series of α-P-boronated ATP derivatives (ATP-α-B) with an improved stability - compared to the natural agonist - was synthesized by substitution of a non-bridging O with a borane (BH3) group. This introduces a chiral center resulting in pairs of diastereoisomers. Due to the high water solubility and the flexibility of the molecule an absolute configuration is yet to be assigned to them. The two diastereoisomers are distinguished as A- and B-isomers (2). The structural differences of these diastereoisomers, however, are expected to cause them to exhibit varied actions. This difference in activity could be used as a tool to investigate the structural differences in the P2Y receptors. Thus, we tested their effects on P2Y1- and P2Y2-receptors. HEK 293 cells stably expressing the rat brain P2Y1-receptor (rP2Y1), and the

rat lung P2Y2-receptors were used. Both receptors were attached with GFP (rP2Y1-GFP, rP2Y2-GFP). Two functional assays were carried out: 1) determination of Ca^{2+} release and 2) agonist-induced receptor translocation due to endocytosis.

2-MeS- and 2-Cl-substitutions on ATP-α-B (A-isomers) increased the potency to induce Ca^{2+} release up to 100-fold, with EC50 values of $4.5x10^{-9}$ M and $2.6x10^{-9}$ M compared to that of the unsubstituted ATP-α-B. Importantly, all A-isomers of ATP-α-B analogues were more potent than the corresponding B counterparts, with a 20 fold difference for 2-MeS-ATP-α-B isomers. In accordance to these functional results obtained with Ca^{2+} release, agonist-induced receptor internalization, visualized by GFP fluorescence, is induced more pronounced by the 2-MeS-substituted derivatives as compared to the 2-Cl-substituted derivatives. Likewise, A-isomers are more potent in this respect in comparison to the B-isomers. Stimulation of Ca^{2+} release and promoting receptor internalization at rP2Y2-GFP with these agonists revealed that they are very weak agonists here in comparison to ATP. At 10 μm concentration these agonist did not induce any internalization of rP2Y2GFP and at 1 μm there was no release of Ca^{2+}. In case of rP2Y1 these compounds induced at these concentrations almost complete receptor internalization and a Ca^{2+} release, which was around 85% of the maximum response. Therefore, we conclude that the new agonists may be considered as potent and specific drug candidates for P2Y1-receptors.

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222 Morphology does not help comprehension in agrammatism: A study of German and Hebrew

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Agrammatism in Broca's aphasia is a language disorder that is characterized by a selective deficit in syntax, both in comprehension and in speech production. According to one of the leading theories for agrammatic comprehension, the Trace Deletion Hypothesis (TDH, Grodzinsky, 1990, 1995, 2000), the deficit in comprehension is attributed to impaired representation of structures with non-canonical word order that are derived by syntactic movement.

The central research question of our study was whether this generalisation holds in languages that have richer morphology than English. The generalisability of the TDH to morphologically rich languages is not obvious, given that case, number or gender morphology can provide explicit cues to the detection of the Agent in a sentence and thus might help the patient to decide who is doing what to whom in a sentence comprehension task.

Data will be presented from a study that investigated the syntactic comprehension of seven German-speaking and six Hebrew-speaking agrammatic subjects. Compared to the English language in which most studies on agrammatic sentence comprehension are done, German and Hebrew have the clear advantage of allowing the study of the inter-

action of syntactic changes of an underlying word order by movement of constituents (e.g. topicalisation) and morphological devices (e.g. case, number and gender inflection) in the interpretation of sentences. Furthermore, due to their relatively free word order, German and Hebrew allow the direct comparison of strictly minimal pairs of canonical SVO and non-canonical OVS sentences in addition to the rather controversial active-passive contrast which underlies many studies.

The studies used a binary sentence-picture matching task, with pictures that either matched the sentence or included reversed thematic roles.

In German five left hemisphere CVA-patients and five normal controls participated in the study. All patients had agrammatic spontaneous speech. The task was to point to one of two pictures that matched a sentence heard before. A total of 264 items including several sentence constructions were tested such as declaratives and relatives, each both in the canonical and non-canonical word order. In addition to sentences with unambiguous case or number morphology, case and number ambiguous sentences of the type NVN (n=22) that allow either a canonical or a non-canonical reading were used as a baseline to see whether morphology makes a difference.

In Hebrew 6 agrammatic aphasics with agrammatic spontaneous speech participated, and 6 normal controls. The task was binary sentence-picture matching, in which the patient heard a sentence and had to choose between two pictures – one matching the sentence, the other including the reversed roles. A total of 777 sentences were included in the study that tested topicalization structures (OVS and OSV) as well as subject and object relatives. The gender of the agent and the patient was manipulated – in the gender-marked sentences, the agent and patient differed in gender, thus allowing the verb to give a hint of gender inflection, in the non-gender marked sentences, both agent and patient were of the same gender, and thus verb inflection could not provide a clue for comprehension.

The results of this study in both German and Hebrew indicate that morphology does not assist interpretation given a deficit in the comprehension of syntactic movement. In German, case and number agreement did not improve comprehension of non-canonical sentences, in Hebrew gender agreement did not improve comprehension. Thus, our data confirm the TDH predictions for the group of agrammatic subjects for case and number marked sentences as well as gender marked sentences.

Processing sentences with and without movement of phrasal constituents – an event related fMRI study

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INTRODUCTION: A current neurolinguistic theory of syntactic comprehension disorders in agrammatic aphasic patients the Trace Deletion Hypothesis postulates a specific impairment in processing syntactic chains, and that this function is mediated by Broca's area. It is supposed that agrammatic aphasic patients are specifically impaired in understanding movement-derived sentences (e.g. passives, object relatives and object clefts) as compared to sentences without movement (e.g. actives, subject relatives or subject

clefts). Thus a different neuronal organization of these two kinds of syntactic processing and a specific engagement of Broca's area in processing moved sentences is suggested. The aim of this study was to investigate whether the specific involvement of Broca's area in processing syntactic traces can be verified using functional brain imaging.

METHOD: We used event-related functional magnetic resonance imaging (fMRI, 1.5 T Siemens Vision) while 13 healthy, right handed subjects were asked to judge the grammaticality of visually presented German sentences with and without movement of phrasal constituents. The sentences were either correct or contained a grammatical error. All sentences were semantically plausible and orthographically correct. They were matched across conditions for length and word frequency. The participants were instructed to intuitively respond to every sentence by pressing one of two buttons with the left hand as quickly and correctly as possible.

RESULTS: Behavioral data showed no differences in reaction time and accuracy between moved and nonmoved sentences. During both sentence conditions fMRI showed activation in language related brain regions. Comparing both kinds of sentences did not result in differential brain activation of left frontal or temporal lobes. In particular, Broca's area was similarly activated in processing both moved and nonmoved sentences. Preliminary results using auditory stimulus representation showed a similar pattern of activation in language related areas as visually presented sentences.

DISCUSSION AND CONCLUSION: Since there were no differences in reaction times and accuracy of reactions between the conditions, difficulty/processing load would seem to be equal in all conditions. In this study processing of different grammatical sentence types did not result in the expected specific activation of Broca's area. Our results rather showed very similar patterns of cortical activation in Broca's as well as Wernicke's area. Thus, Broca's area seems to be involved in general syntactic processing as required by grammaticality judgments rather than having a specific function in transmitting syntactic relations.

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224 The effect of emotionally loaded distracters on neural activity ERP study of a cued attention task with verbal distracters

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Rationale: the main purpose was to study the unique effect of emotionally loaded stimuli on unrelated cognitive (neural) activity. The study aimed to explore the general effect of emotionally loaded stimuli on brain activity, beyond the direct ERP locked to the loaded stimuli themselves.

Auditory cued attention was studied lately using ERP, but the effect of distracters given during the task has never been studied. ERP recording during cued attention task with

emotionally loaded distracters, allow studying the neural basis of attentional and emotional processes.

Methods: Ten subjects performed an auditory cued attention task, while EEG was recorded. The cued attention task involved presentation of a cue stimulus which provided information to direct attention to the following target stimulus to which the subject was to respond. In the minority of cases, the cue provided erroneous information about the target. During the task, in one third of the rehearsals, a first name was heard as a distracter between the cue and the target. The affective valence of each first name was determined following the experiment, during a thorough interview. Evoked potentials were averaged according to 2 dimensions: Cue validity, and the affective valence of the distracter. Trials with no distracters were averaged separately.

Results: The distracters' effect on the neural response to the targets (tone stimuli) was modulated by the affective valence of the distracters. The effect lasted at least 2 seconds following the onset of the distracters. There was interaction between this effect and the cue validity effect on the brain response to the target.

Conclusions: There is a unique effect of emotionally loaded verbal stimuli on unrelated cognitive activity lasting for seconds. This effect can be explored using evoked potentials.

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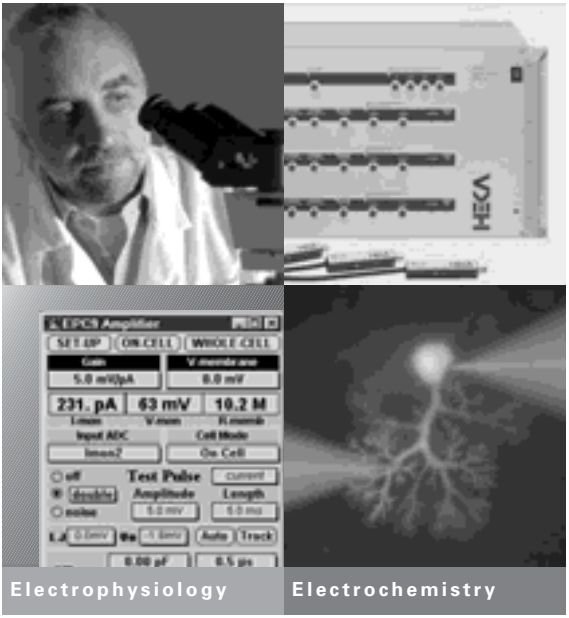

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Introductory Remarks to Symposium 24

Attentional modulation of sensory information processing in man and monkey

Stefan Treue

The visual system of man and other highly evolved animals supplies a wealth of detailed information about the visual environment. Yet at any given moment much of this information is behaviorally irrelevant. If evolution would not have also endowed the nervous system with mechanisms to control the flow of information, only a small fraction of our processing capabilities could be devoted to critical aspects of the incoming sensory signals. In addition to bottom-up mechanisms the visual system uses attention as a powerful top-down influence to optimize the use of its processing resources by allowing us to concentrate processing on a very small proportion of the incoming information.

In recent years a combination of psychophysical and functional cortical imaging studies in humans, with computational modeling and electrophysiological recordings in non-human primates have brought an explosive growth in our understanding of the mechanisms and perceptual effects of attention.

Such studies have shown that the allocation of attention enhances the processing of attended locations or stimulus features and suppresses those from unattended locations or features. The effect of such attentional selection is dramatic, leading to severe perceptual deficits for unattended aspects of visual scenes and playing a crucial role in the control of goal-directed movements (H. Deubel). Psychophysical experiments in humans have helped to quantify the perceptual effects of attention, providing the constraints needed for realistic models of attentional mechanisms (J. Braun). Cortical imaging techniques have elucidated the networks of cortical areas that underlie the deployment of attention (S. Kastner). Single cell recordings in awake behaving monkeys have provided important information as to where and how the interaction between bottom-up sensory signals and top-down influences takes place (P. Fries, S. Treue). Attentional influences have now been demonstrated throughout visual cortex but with an increase as one ascends the hierarchy of visual areas in primate cortex, ultimately resulting in a neural representation of the visual world that is dominated by the behavioral relevance of the information rather than being primarily designed to provide an accurate and complete description of it.

The symposium will give an overview of the state-of-the-art in attentional research covering a range of approaches, but all aimed at a central area of research in cognitive neuroscience.

225 Attention and awareness in goal-directed eye and hand movements

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In recent years, much behavioural and physiological evidence has accumulated that when an eye or a hand movement to a goal is prepared, visual attention is central for the selection of the movement target among the competing visual elements. In the first part of the presentation, experiments will be presented that extend these basic findings by investigating covert selective processing in more complex movement situations, i.e., for sequential eye and hand movements directed to two targets, for reaching movements around an obstacle, and for grasping an object. In all these experiments, a dual task paradigm was applied which combines movement preparation with a perceptual discrimination was applied. The experimental results demonstrate that the paradigm is well suitable for studying the dynamic deployment of attention in the movement preparation phase. Some interesting differences between the attentional selection for eye and hand movements, respectively, will be discussed.

To what extend are people aware of what target they select and fixate at each moment in time? This question is addressed in the second part of the talk. Experimental evidence will be presented suggesting that people don't always know what they are looking at, or at least, when they are looking where. We believe this occurs because shifts of visual attention are mistaken for shifts of the eyes. The data suggest that as soon as 100-250 ms before the saccade, "subjective gaze" moves from the fixation to the target with a speed of about 20 deg/sec and in an analogue manner, covering intermediate spatial positions.

226 The physiology of attention in the "where" pathway: Location, features and objects

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Information processing in primate visual cortex occurs primarily along two pathways. These pathways are each a hierarchical succession of cortical areas specialized for the analysis of particular aspects of visual information. They both start in primary visual cortex (V1). As one moves up these pathways to higher cortical areas the receptive fields of the neurons increase in size while the neurons become selective for increasingly complex aspects of the visual information. The pathway leading to the temporal lobe contains neurons specialized for the processing of such visual attributes as orientation, color and form and is correspondingly often referred to as the “what” pathway. The pathway leading to the parietal lobe contains neurons specialized for the processing of motion, stereoscopic depth and spatial layout of the environment and is therefore often referred to as the “where” pathway.

While studies of primate visual cortex have originally concentrated on sensory aspects of information processing the last decade has brought a shift to investigations aimed at an understanding of the influence of cognitive, higher level factors, most notably attention. While attentional influences in early areas of the temporal pathway were recognized early on, such influences on processing in the dorsal pathway were clearly established only more recently.

The presentation will provide an overview of these investigations, concentrating on attentional influences on the processing of visual motion information derived from electrophysiological recordings from the dorsal ("where") pathway in macaque monkey visual cortex. The animals were trained to attend to one (the target) of several moving stimuli on a computer monitor and to respond to changes in the target, ignoring changes in the other stimuli (the distractors). At the beginning of each trial the animal was instructed as to which stimulus would be the target in the upcoming trial. Sensory stimulation was the same in all trials but which stimulus was designated the target varied. This allowed assessing the effect of different attentional conditions.

The results demonstrate a profound influence of attention on neuronal firing rates. Furthermore the data demonstrate that attentional modulation is multiplicative, preserving the selectivity of the neurons, but increasing the influence of attended and reducing the influence of unattended stimuli. They show that attentional modulation can be based on the attended location as well as the attended stimulus feature. Comparisons to the modulation evoked by stimulus contrast show a high degree of similarity between the two influences on a sensory neuron's response that suggests common mechanisms.

These results show that attention has a powerful and systematic influence on the processing of sensory information in the dorsal visual pathway of primate cortex and that even at rather early stages of visual information processing neural signals do not only reflect the sensory characteristics of the neurons but also the behavioral relevance of the stimuli inside the receptive field.

The physiology of attention in the "what" pathway: Oscillatory neuronal synchronization and firing rates

227

Pascal Fries

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If two high contrast stimuli are present in the receptive field (RF) of an extrastriate neuron, its firing rate is dominated by the attended stimulus. However, attention has much less effect on firing rate if the second stimulus is outside of the neuron's RF. This raises an apparent paradox. RFs grow larger at each successive stage of visual processing. Thus, two stimuli located in the RFs of separate neurons at one stage will both be contained in the RF of one neuron at a subsequent stage. How is the response of the postsynaptic neuron with a large RF biased in favor of the attended stimulus, when there is no change in firing rate of the presynaptic neurons with smaller RFs? We hypothesize that attention causes the first stage neurons with small RFs to fire synchronously to the attended stimulus, thereby having a larger impact on postsynaptic neurons with large RFs. We recorded spikes from single cells and local clusters of cells, as well as the local

field potential (LFP) from multiple electrodes in V1, V2 and V4. RFs in each area were overlapping. Neurons were stimulated with patches of moving high contrast square wave gratings. One patch appeared in the region of RF overlap while the other was at the same eccentricity in another quadrant. The monkey was cued to attend to one stimulus and had to detect a change of its color occurring at a random time, while ignoring color changes in the other stimulus. In all three areas, local synchronization was assessed by means of spike-field coherence (SFC). The SFC in all areas was strongly dominated by a broad peak between 40 and 70 Hz, the γ-frequency range. When attention was directed to the stimulus in the RF of the recorded neurons, oscillatory neuronal synchronization was modulated in a majority of recording pairs but to different degrees in the different areas. In area V1, individual pairs of recording sites showed significant increases or decreases in γ-frequency synchronization, but there was no significant net effect across the population. In V2, γ-frequency synchronization on the population level was slightly but significantly enhanced and coherence was slightly shifted to higher frequencies. In V4, γ-frequency synchronization was strongly enhanced over the entire γ-frequency range. Increases in γ-frequency synchronization were typically accompanied by decreases in low-frequency synchronization. We conclude that increased γ synchronization and reduced low frequency synchronization magnifies the efficacy of signals from attended stimuli, providing a possible mechanism for how attention biases the competition between multiple stimuli in one RF.

228 Mechanisms of visual attention in the human brain

Sabine Kastner

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Attentional mechanisms are needed to select relevant and to filter out irrelevant information from cluttered visual scenes. There is converging evidence from single-cell physiology studies in monkeys and functional brain mapping studies in humans that selective attention modulates neural activity in visual cortex by facilitating neural responses to attended stimuli, by suppressing neural activity to ignored stimuli, and by elevating neural baseline activity in the absence of any visual stimulation. Our recent findings indicate that these attentional effects are not confined to cortical processing, but occur already at the level of the lateral geniculate nucleus. Selective attention appears to increase neural response gain at the thalamic level. At intermediate cortical processing levels and attention appears to use intra- and extra-RF mechanisms to filter out irrelevant information. Experimental evidence will be presented to support a “push-pull” model of selective attention that integrates the various attention effects found in the visual system. These signals appear not to be generated in the visual cortex or in the thalamus, but in a distributed network of higher-order areas in frontal and parietal cortex that are involved with attentional control.

Attention as a bottom-up process 229

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Attention gives us the freedom to perceive one and the same stimulus in different ways. A closer look shows, however, that this freedom is limited by stimulus-driven, or bottom-up processes. I illustrate this fact with three psychophysical findings: (i) the attentional demand of disparate discriminations is essentially the same, (ii) withdrawing attention does not prevent the conscious perception of salient stimuli ("vision outside the focus of attention"), and (iii) the attentional modulation of spatial filter populations seems to depend on stimuli, not task, so that attention may at times be counterproductive (i.e., reduce performance). These psychophysical results agree broadly with 'biased-competition theories' of attention. However, several puzzles remain, amongst them how the 'limited capacity' of attention can emerge from a highly parallel neural architecture.

The Role of the Primate Area MT in Manual Tracking Tasks 230

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It is widely accepted that the STS-region of the primate is involved in the perception of moving visual stimuli and in the control of visually guided eye movements (saccades and smooth pursuit). We are interested if and how this region is related to the control of visually guided arm movements as well. We trained rhesus monkeys to control a cursor on a screen by means of a two dimensional manipulandum and to perform tracking movements with moving targets, while keeping their gaze on a fixation point on the screen.

In a first series of experiments we compared the activity of medio temporal area (MT) neurons in "Tracking" (motor) and "Replay" (visual) conditions to see if there is any extraretinal modulation of the neuronal activity during these kinds of tasks. In "Trakking" conditions the monkey performed visually guided manual tracking movements in 3 different directions centered around the preferred direction of a MT neuron. In the "Replay" conditions the monkey held his arm stationary and the identical visual scene of the "Tracking" conditions, including fixation point, target and (replayed) cursor movement, was repeated. In a sample of 38 (multi/single) units of 1 monkey, we found 18 (47%), that showed no significant difference in the neuronal activity between "Trakking" and "Replay" conditions. 11 (29%) of the units fired at higher rates in the "Trakking" and 9 (24%) in the "Replay" conditions. The significant modulations of the activity in half of the cells shows, that the behavioural context of this kind of task is reflected in the activity of MT neurons.

In a second series of experiments we perturbed MT activation by electrical microstimulation, while the monkey performed a "Bend-Tracking" paradigm. In this paradigm the target initially moved linearly downwards and then bent its trajectory to 1 of 4 directi-

ons. This change in direction coincides in time with the onset of stimulation current and with the target entering the center of the receptive fields at the site of stimulation. By comparing the trajectories of the monkeys hand in stimulated and non-stimulated trials, we found two main effects: 1) The reaction time to the change of target direction significantly increased. 2) The direction of bend reaction was affected by microstimulation, in some cases in favour of the preferred direction of the stimulation site. Both results indicate the monkeys difficulty to guide his hand by ambiguous motion information from area MT.

Together these findings support the view that area MT is involved in the network that performs visuomotor transformations for limb movements as well.

231 Attentional and Sensory Influences on Visual Motion Detection and Discrimination thresholds

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Traditionally, basic visual motion processing has often been viewed as taking place without the aid of attention. However, work in the last decade has cast doubt on this view by demonstrating that attention does influence motion processing. In addition, recent work by Hol and Treue (2001) has shown that motion discrimination and detection engage different neural mechanisms. We investigated whether these two basic perceptual abilities also differ in other aspects by comparing their susceptibility to attentional and sensory influences. In order to assess the effects of attention on motion detection and discrimination we compared single task against dual task performance in a 2-AFC paradigm using moving random dot patterns as stimuli. Sensory influences, on the other hand, were determined by conducting these two tasks at different levels of contrast. In our detection task, participants had to detect coherent horizontal motion and thresholds were obtained by determining the percentage of dots that were needed to make a correct judgement. In our discrimination task, participants had to discriminate small deviations from vertical motion and thresholds were obtained by determining the degrees of deviation yielding correct judgements. The detection stimulus and the discrimination stimulus were of the same contrast and were always presented simultaneously for 235 ms to the left and right of a central fixation point. In a focused attention task (single task) subjects had to perform either the detection or the discrimination task and were told to ignore the other stimulus. In a divided attention task (dual task) subjects had to perform both tasks concurrently. Our results show that both detection and discrimination thresholds are strongly modulated by attention: Thresholds are higher in dual tasks than in single tasks. Remarkably, contrast manipulation showed opposite effects on detection and discrimination performance: While detection thresholds are lower with lower contrast, discrimination thresholds are higher with lower contrast. Taken together, the outcome supports the existence of separate neural mechanisms for detection and discrimination of visual motion.

Visual Spatial Attention Modulates Erp Brain Responses to Mislocated Task-Irrelevant Tones in the Ventriloquism Illusion 232

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To investigate attention effects on audio-visual integration during the ventriloquism illusion, we displayed two randomly intermixed streams of unilateral visual stimuli, half accompanied by a synchronous, task-irrelevant, binaural tone presented frontally. The ventriloquism effect led to illusory mislocation of these central tones towards the concurrent lateral visual stimulus. In different blocks, subjects attended to either the left or right visual streams to detect infrequent visual targets. Attentional modulation of ERPs to visual-alone stimuli included P1 (90-130ms) and N1 (150-200ms) effects. To assess attention effects on the ventriloquized tones, we first subtracted the ERPs to visual-alone stimuli from the ERPs to the corresponding audio-visual events (presented on the same side and thus under the same attention condition), thereby removing the overlapping visual components and visual attention effects. Contrasting such difference waves for tones integrated with an attended object vs. tones integrated with an unattended object revealed the role of attention in this crossmodal grouping.

Although all tones were task-irrelevant and came from the same unattended central-space location, tones paired with attended visual objects elicited an enhanced prefrontal negativity from ~220-700ms poststimulus. These results thus suggest a late attentional selection or capture of the central task-irrelevant tones due to their cross-modal grouping with the simultaneous attended visual object. This view was further supported by a second experiment that included central tones presented alone (and thus not mislocalized). ERPs to tones coupled to unattended visual stimuli fell in between ERPs to tones coupled to attended visual stimuli and ERPs in the tone-alone condition.

Spike frequency adaptation may explain attentional effects in visual neurons 233

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It is well established that visual attention increases the responses of single neurons to behaviorally relevant stimuli, but little is known about the underlying mechanisms of this modulation. Furthermore, adaptation effects are typically larger at later stages in the visual hierarchy. We recorded single cell responses in areas MT and MST of two macaques while the animals attended or ignored a moving stimulus located inside the receptive field. Here we present data showing that: 1) MT and MST contain both tempo-

rally sustained and transient units, both of which show attentional effects; 2) attentional effects grow linearly over time as a function of the stimulus presentation time in MT and MST and 3) the magnitude of attentional modulation averages 15% in MT and 31% in MST. These results can be explained by the hypothesis that adaptation results from a decrease in spike frequency adaptation of cortical neurons under neuromodulatory control (Wilson et al., 2000). To show this, we modeled the results by connecting model (sustained and transient) MT neurons to model MST, with each model neuron possessing a slow feedback mechanism that caused spike frequency adaptation. By introducing the same attentional reduction in adaptation at both the MT and MST levels, all of the data could be satisfactorily predicted. In particular, the increased attentional effect in MST was shown to result from the product of equal local effects in both MT and MST. This supports the hypothesis that selective neuromodulatory control of spike frequency adaptation in single neurons throughout the visual hierarchy is the mechanism by which attention modulates neuronal responses in the cortex.

234 Neural mechanisms of conflict-triggered inhibition of distracting perceptual dimensions during task-switching

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Adaptive human behavior requires a context-sensitive balance between flexible adjustments of cognitive sets in response to changing task demands on one hand and stable maintenance of cognitive sets in face of distraction, on the other. The aim of the present experiment was to explore the neural mechanisms that enable humans to meet these antagonistic requirements on action control. 12 healthy right-handed volunteers underwent fMRI while performing a task-switching paradigm, in which abstract objects had to be classified according to either color or shape. Response conflicts were induced by the occurrence of incongruency in the irrelevant stimulus dimension. In addition, a working memory task was conducted in order to identify prefrontal brain regions as well as parietal and occipitotemporal association areas involved in the mnemonic processing of the stimulus dimensions used in the task switching experiment, i.e. color and shape. Preprocessing of the functional images used SPM99 and comprised corrections for slicetime acquisition differences, motion artifacts and low frequency fluctuations, coregistration, normalization into standard stereotactic space and spatial smoothing. For group statistics, random effects analyses were performed on single subject contrast images. We found reliable switch costs as well as a significant effect of response incongruency of the irrelevant stimulus dimension both in the color and in the shape tasks (incongruent trials: 545.16ms, congruent trials: 522.35ms; $p<0.01$). Response incongruency was associated with reliable activation of the medial frontal cortex adjacent to the anterior cingulate cortex (BA 8m), but not of the anterior cingulate cortex itself. Furthermore, probably due to the fact that in our paradigm most of the stimuli had a neutral color (white), the rare occurrence of red or blue color during shape tasks produced a mismatch-like effect with prolonged reaction times and enhanced activity in a network of frontal, parietal and occipitotemporal brain areas. Similar cortical regions were also involved in the memory task for shapes. Hence, we assume that these brain regions subserved top-down attentional control processes directed to the task-relevant shape.

Apparently, these processes were enhanced by rare, but salient changes of sensory (i.e. color) information outside the current focus of attention, probably because such changes of the environment could be relevant for adapting the behavior. Further analyses will determine the functional connectivity between the activated brain regions in order to assess the dynamic interactions of bottom-up and top-down processes involved in this task-switching paradigm.

235 Components of an antennal mechanosensory pathway in the stick insect

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Stick insects (*Carausius morosus*) move their antennae during locomotion (Dürr et al. 2001, J.Comp.Physiol.A 187), employing them as active tactile sensors to guide front leg movements. In the present study, three steps were taken to identify components of the neural pathway underlying the sensorimotor link between mechanosensory hair plates of the antennal scape-pedicel joint (pHP) and the prothoracic ganglion (T1). First, afferent projections of the ventro-lateral and dorso-medial hair plates at the base of the pedicel (pHPvl and pHPdm, resp.) were traced into the deutocerebrum (DC). Second, neuron clusters with neurites descending to the T1 were backfilled via the neck connective or the first thoracic connective. Third, intracellular pioneer recordings in the DC yielded a first sample of candidate neurons that convey descending antennal mechanosensory information about movement of the pedicel relative to the scape.

Afferent projections of the two pHP types revealed characteristic differences. Whereas the arborisations of pHPdm afferents were marked by conspicuous, nearly parallel dorsal neurites that branch off the main tract, pHPvl afferents lack these neurites, but show a dense dendritic network with a bundle of dorso-rostral branches. Both afferent types have a ventro-medial bundle of neurites running parallel to the medial border of the DC. pHPdm afferents typically terminate in the suboesophageal ganglion, whereas pHPvl afferents project to the T1. Both Co^{2+} and Neurobiotin stains of pHP afferents showed dye-coupling with up to 10 neurons. The most frequently observed dye-coupled neurons had their somata in the ventro-lateral rind of the DC. pHPvl afferents were generally dye-coupled to more neurons than pHPdm, including a neuron with a soma in the midline region of the protocerebrum.

Connective backfills labelled both ipsi- and contralateral interneurons in the brain. Their somata could be assigned to one of three clusters per hemisphere, or lay close to the midline of the protocerebrum. The purely ipsilateral cluster A was located in the ventral rind of the protocerebrum. Bilateral clusters B were of elongated shape, extending diagonally from the anterior and ventro-medial to the posterior and dorso-lateral protocerebrum. Bilateral clusters C were located in the ventro-medial DC, ventral to a commissure that contained most of the axons of the contralateral neurons.

Intracellular recordings revealed two ipsilaterally descending interneurons which responded to ramp-and-hold stimuli applied to the scape-pedicel joint. One interneuron

had its soma in the ventral rind of the protocerebrum close to the midplane of the brain. Its dendritic arborisations were restricted to the dorso-anterior DC. This interneuron responded to any deflection of the scape-pedicel joint throughout an 30° wide angular range around the resting position of the joint. The soma of the second interneuron lay in cluster C. This interneuron also ramified in the dorso-anterior DC with 2 branches extending into the posterior protocerebrum. Forced deflections of the pedicel elicited compound EPSPs and spikes, with maximum responses to movement in an 8° wide angular range medial to the antennal resting position in. The projection areas of both interneurons posterior to the brain are unknown.

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236 How are the rainbow trout's pretectal direction-selective neurons involved in the optokinetic reflex?

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In a sub-region of the rainbow trout's pretectum we identified direction-selective neurons in electrophysiological experiments. These direction-selective neurons encode horizontal, vertical, left-anterior to right- posterior and right-anterior to left-posterior moving visual stimuli and thus may subserve similar function in optokinetic eye-movements (OKN) control as the nucleus of optical tract and dorsal terminal nucleus (NOT, DTN) for horizontal- and the medial terminal nucleus (MTN) for vertical optokinetic eye-movements in mammals. To support this theory we analysed the effects of lesioning the recording site of these direction selective pretectal neurons on the slow-phase of optokinetic eye-movements in trouts.

The behavioural response was exactly measured using the search coil technique. The visual stimulus was a black and white random dot pattern inside of a rotating drum. The fish were fixed centred in a water filled plexiglas tank. Left eye was covered and the visual stimulus moved in naso-temporal (N-T) and temporo-nasal (T-N) direction with velocities of 4, 9, 14 and 18°/s. In the electrophysiological experiments the fish was anaesthetised and immobilised. Visual stimulation was a random dot pattern projected by a planetarium inside a water filled perimeter with a velocity of 10°/s. We tested four rotations axes of the planetarium, horizontal, vertical, left-anterior right- posterior and right-anterior left-posterior. In every position the visual stimulus turned in clockwise and counter clockwise direction. Single-unit recordings were made with a glass coated tungsten microelectrode.

First we tested 6 rainbow trout's horizontal monocular OKN (hmOKN) in behavioural experiments. In five fish we located the direction-selective neurons in the left dorsal part of the pretectum by recording their characteristic discharge pattern to optokinetic stimulation, made lesions by applying 10 mu A for 60 sec through the recording electrode and subsequently investigated the hmOKN. One fish was the control without lesion but the same number of penetrations in the surrounding area.

Before the lesion all tested trouts showed a normally hmOKN. The gain in T-N direction was 1.2 and N-T 1.15 at 4°/s stimulus veloscity. By increasing the stimulus velocity (until 18°/sec) the gain dropped linearly to 0.45 in T-N and 0.3 in N-T direction. After micro lesions at three different rostro-caudal positions in the pretectum, the hmOKN in all five fish was affected. One fish (lesion caudal) showed a strong reduction of the gain to ipsiverive stimulus direction. In another fish (lesion midrange) the gain declined in contraversive and ipsiversive stimulus direction. In three other fish (lesion rostral) the gain in contraversive stimulus direction went down to 0.05. The control fish was without deficit in optokinetic eye-movements. These results indicate that contrary to mammals direction-selective neurons in one pretectal hemisphere of the rainbow trout encode ipsiversive as well as contraversive stimulus directions presented to the contralateral eye and thereby the ipsiversive and contraversive eye velocity in the slow phase of optokinetic eye-movements.

Serum and glucocorticoid-regulated kinase: A differentially expressed gene in a MPTP-model of Parkinson`s disease 237

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While many genes associated with Parkinson`s Disease (PD) have been studied, the multifaceted and complex pathophysiology of the disease has resisted a comprehensive understanding of its progression. We have used our proprietary automated PCR-based procedure, the digital expression pattern display (DEPD®) technology (patent: DE198 06 431, PCT/EP99/00992), to analyse the gene expression profile after an acute 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) -injection into 1y-old mice (Stichel-Gunkel et al. 2002, Soc.Neurosci.Abstr.32:595.1). Among the large number of differentially expressed genes, the serum and glucocorticoid-regulated kinase (sgk) was one of the strongest regulated genes in the substantia nigra (SN).

The present study was designed to (i) to validate the differential expression (ii) to identify the cells that upregulate sgk and (iii) to correlate the time point of upregulation with histopathological changes in the animals.

Therefore we treated 1y-old C57BL/6 mice with MPTP (15 mg/kg; 2x/d, 2d, total cumulative dose of 60 mg/kg; i.p.) or saline and analysed the sgk expression at various time points after treatment using non-radioactive *in situ* hybridization (ISH). To generate a negative control some animals were pretreated with the monoamine-oxidase inhibitor selegiline (15mg/kg; i.p.).

Upregulation of sgk-transcript was confirmed by ISH with DIG-labeled riboprobes. Three hours after MPTP application an increase in staining intensity and number of labelled cells were noted in the SN as well as in other brain regions (cortex, hippocampus, thalamus) of MPTP-treated animals. Histopathological analysis showed that this upregulation is accompanied by a significant cell death of nigral neurons. The fast sgk-upregulation was followed by a gradual decrease to baseline levels at one week after treatment. Colocalization studies with ISH followed by immunhistochemistry with

neuron-specific antibody NeuN or astrocyte-specific antibody glial fibrillary acidic protein revealed sgk transcript in dopaminergic neurons, astrocytes and oligodendrocytes. The upregulation of sgk transcription was prevented by selegiline pretreatment.

Our results revealed a gene, that is strongly regulated during MPTP-induced pathogenesis. The positive correlation of sgk upregulation and the onset of nigral cell death implies that sgk contributes to the cascade of events leading to cell death. The overall upregulation of sgk, however, suggests that MPP+ excerts a widespread transcriptional response in the brain including also non-dopaminergic cells.

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238 Complex innervation of the second antennal segment of crickets

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In crickets, at least five identified descending brain interneurons convey information about antennal movements to the thoracic ganglia. The origin of the synaptic inputs onto two of these interneurons has been localised to the second antennal segment, the pedicel of the ipsilateral antenna by means of electrical stimulation of single antennal sensory nerves ([1]). Individual proprioceptors synapsing onto the interneurons, however, have not yet been identified. To further narrow down the sources of synaptic inputs, a backfill study was carried out to characterise the proprioceptors located in the cricket pedicel. Lesion experiments to analyse the sensory control of the antennal tracking behaviour ([2]) showed that besides hair plates additional proprioceptors must exist in the pedicel ([3]). Anatomical studies in other insects ([4]) support this result. We show here results of wholemount preparations or serial sections of 25 hexammine cobaltic chloride-backfilled antennae. They reveal different clusters of neuronal somata in the pedicel: a) A group of 13 to 16 large campaniform sensillae (diameters around 10 µm) encircle the distal edge of the pedicel. Axons of these campaniform sensillae project into two separate nerves (lN1a, mN1a) that arise from the lateral and the medial N1 (lN1, mN1) in the distal scape, respectively. b) The ventral side of the pedicel houses a group of 5 to 11 somata with a diameter of 6 to 15 µm. These have a common nerve that branches off from mN1a in the distal scape. The dendrites of these cells point distally suggesting that this soma cluster might represent a chordotonal organ attached to the distal edge of the pedicel. c) A group of similarly sized neurons is located in the ventro-distal part of the pedicel. The nerve of this group originates from lN1a in the pedicel. This organ might also represent a chordotonal organ attached to the distal edge of the pedicel. d) In two preparations, 2 or 4 single neurons, respectively, were observed which have a 7 µm long, spindle-shaped soma, and dendrites with at least 40 µm length which point towards the distal edge of the pedicel. These neurons are equidistantly spaced around the perimeter of the pedicel. The identity and the origin of the supplying nerves are unknown. e) A large number of faintly-stained small neurons (7 µm and smaller) is visible

in all preparations. They appear to take up the whole lumen of the pedicel. We hypothesize that these neurons belong to a Johnston's organ. In summary, multiple proprioceptors reside in the pedicel providing synaptic input to the descending brain interneurons. Electrophysiological experiments are currently being carried out to reveal the specific connectivity of the pedicellar proprioceptors.

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Innervation, distribution and central projections of the paraproctal sense organs in the female desert locust

239

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The left and right paraproct of female locusts are located beetween the dorsal ovipositors, the epiproct and the ninth abdominal sclerite. They are positioned as the most posterior abdominal segment. Relatively little is still known about the function and location of paraproctal sense organs (Thomas 1965, Tousson and Hustert 200) compared with other body segments. Here the distribution and the peripheral innervation of the sensory organs on the paraproct has been studied in whole-mount preparations by using the cobalt and biotin backfill techniques. The paraproct of the female locust bears hair sensilla of three basic types: a) Mechanosensory hairs (bristle or trichoid) each supplied with one sensory cell, b) Dually innervated mechanosensory hairs with a fine cuticular shaft which are restricted to the region near the posterior edges of the outer faces, c) Basiconic hairs which are multimodal receptors which encode both mechanical and chemical contact cues.

The morphology and organization of the central projections of chemoreceptors and mechanoreceptors afferent from the paraproct were examined by biocytin staining individual hair afferents. All afferent fibers project in the tenth neuromere of the terminal ganglion and with their collaterals project further into anterior neuromeres and free abdominal ganglia. Projections from single multiply innervated hair sensilla do not segregate with the exception of one afferent of contact chemosensory hairs which terminates only in its segmental neuromere, as was shown for other contact chemoreceptors of the abdomen (Tousson and Hustert 2000). We think these sensilla at the very tip of the abdomen play a major role for mating, for the selection of ovipositon sites and during the different oviposition subroutines.

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240 Conduction of Receptor Current through the Sensory Dendrite of a Spider Mechanoreceptor Neuron

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Transduction in mechanoreceptor neurons of both vertebrates and invertebrates usually happens at the ends of fine dendritic processes, spatially distant from cell regions that can be penetrated with microelectrodes. This makes it difficult to estimate the amplitude and time course of the receptor current at the site of transduction. It is also hard to tell if dendritic sensory signal conduction to the soma happens by active or passive mechanisms, and how much attenuation occurs in the dendrite if the conduction is decremental.

The VS-3 slit-sense organ of the spider *Cupiennius salei* possesses relatively large bipolar neurons that can be impaled by microelectrodes to allow current-clamp and voltage-clamp recording during mechanical stimulation (French et al., J. Comp. Physiol. A188: 739-752, 2002). Immunohistochemical studies indicated that voltage-activated sodium channels are present throughout the sensory dendrites of these neurons, at densities comparable to those in the axon, which is clearly excitable. Frequency response measurements of VS-3 neurons also suggested that action potentials are initiated closer to the site of mechanotransduction than to the soma.

We used three approaches to estimate the passive electrical properties of the sensory dendrites in the VS-3 organ: (1) current-clamp measurements of the receptor potential during step mechanical stimuli, (2) current-clamp frequency response measurements of the receptor potential during pseudo-random white noise mechanical stimuli, and (3) voltage-clamp measurements of total charge arriving in the soma during step mechanical stimuli combined with voltage jumps at the soma. All measurements were made by the switching single electrode current-clamp and voltage-clamp techniques with sharp microelectrodes penetrating the neuronal somata. Mechanical displacements were applied to the slits via a servo-controlled piezoelectric stimulator. Tetrodotoxin was applied throughout the experiments and measurements made at the resting potential or more hyperpolarized voltages to eliminate active currents. The results were used to estimate the passive cable properties of the sensory dendrite in conducting the receptor current to the soma, and to test whether normal sensory function requires active propagation along the dendrite.

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241 Extracellular pH Modulates Receptor Current in a Spider Mechanoreceptor

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The molecular basis of mechanical sensitivity in mechanosensory neurons is still unclear. In at least two cases mechanical sensitivity has been associated with acid sensitivity. Murine TREK-1 is a voltage- and mechanically-activated K^+ channel whose mechanical sensitivity is increased by intracellular acidification in the range pH 5-8. The major effect of acid seems to be via open probability but single channel conductance is also affected (Maingret et al., J Biol Chem 274: 26691-26696, 1999). DRASIC, a member of the DEG/ENaC channel family, has been suggested to mediate both touch and noxious acid stimulation in mouse skin (Price et al., Neuron 20: 1071-1083, 2001).

We studied the effects of extracellular pH on the receptor current in sensory neurons of the lyriform organ VS-3, an exoskeletal mechanoreceptor of the spider *Cupiennius salei*. We used the switching single-electrode voltage-clamp technique to monitor the receptor current during mechanical stimulation in an isolated preparation of the intact sensory organ. A perfusion system allowed rapid exchange of extracellular pH. Normal spider saline was buffered at pH 7.8, mimicking the conditions found in the hemolymph of resting spiders. Changes in extracellular pH in either direction modulated the mechanically induced receptor current in VS-3 neurons. Alkaline pH decreased, and acidic pH increased peak receptor current. We performed noise analysis of the receptor current to examine the effects of pH on open probability and single channel conductance of the spider MACs (mechanically activated channels, and found no significant change in the MAC single channel conductance in the tested pH range (pH 10 - 5). However, the channel open probability of the MACs was significantly increased by acidic extracellular pH <6.

Our results show that mechanically-activated channels of spider sensory receptors are modulated by acid. Previous studies on the lyriform organ demonstrated that MACs in VS-3 neurons are Na^+- selective, and can be blocked by amiloride or Gadolinium. All these properties are common features of membrane channels of the DEG/ENaC channel family, suggesting that the spider lyriform organ VS-3 may use mechanically-activated ion channels that are related to the DEG/ENaC family.

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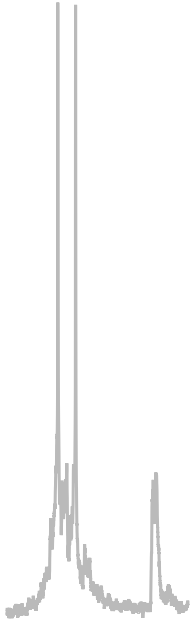

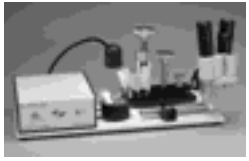

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242 How addition of nitrous oxide to isoflurane anesthesia affects sensory processing in rats

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Nitrous oxide is generally used to supplement inhalational anesthetics because of its presumed analgesic potency. However, when activity of CNS neurons is measured in animal experiments, e.g. to study processing of information about sensory stimuli, the effects of the anesthetics used have to be considered. Unfortunately, notwithstanding the recent enormous increase of knowledge about cellular and subcellular mechanisms of anesthetic actions gathered from *in vitro* studies, it is still largely unknown where and how anesthetics act in the living organism. We studied sensory processing of noxious stimuli (radiant heat applied to the skin) by extracellular recordings of neurons in the posterior complex of the somatosensory thalamus.

Adult rats were anesthetized with the inhalational anesthetic isoflurane and artificially ventilated. Physiological parameters (arterial pCO_2, mean arterial blood pressure, heart rate, rectal temperature) were kept in normal ranges. The potency of inhalational anesthetics is described by the MAC value, where 1 MAC is the minimal alveolar concentration necessary to suppress the movement response to skin incision in 50% of subjects.

Under baseline isoflurane anesthesia (~1.2 vol.%, 0.9 MAC), noxious stimulation elicited excitatory responses (18.2 ± 4.0 spikes/s, mean ± SEM) in 21 thalamic neurons. Addition of 66 vol.% nitrous oxide to baseline anesthesia (resulting in 1.5 MAC) had variable effects on nociceptive responses: in 7/21 (33%) neurons the noxious stimulus still induced an excitatory response (16.1 ± 5.5 spikes/s) similar to that under baseline anesthesia, while the response was abolished in 13/21 (62%) neurons and inhibited in one case. Increase of isoflurane concentration (~1.9 vol.%) to achieve a concentration equipotent to that of the isoflurane/nitrous oxide mixture (1.5 MAC) resulted in a complete suppression of nociceptive responses in all neurons tested (19/19, 100%). Reduction of isoflurane (~0.8 vol.%) in the nitrous oxide/isoflurane mixture to achieve a total concentration equipotent to 1.4 vol.% isoflurane alone (1.2 MAC) again produced different results: with nitrous oxide, 8/14 (57%) neurons showed an excitatory response (30.5 ± 12.3 spikes/s) greater than under baseline anesthesia, 4 (29%) showed an inhibitory response and two were suppressed; under 1.4 vol.% isoflurane, the nociceptive responses were abolished in 9/10 (90%) neurons and only one showed a slight discharge increase. The initial excitatory responses recovered in all cases after return to baseline anesthesia.

The results show that equipotent concentrations of isoflurane and nitrous oxide/isoflurane mixtures have divergent effects. While the increases of isoflurane concentration above baseline induced suppression of the nociceptive responses, i.e. had antinociceptive effects, addition of nitrous oxide had no antinociceptive effect in 33% of the neurons at 1.5 MAC or even facilitated nociceptive responses in 57% of the neurons at 1.2 MAC.

Motor task reduces pain evoked cortical activity: A combined EEG-MEG study 243

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Human cortical activation due to transient painful stimuli is commonly investigated using simple test conditions. However, it is known that psychological factors, sensory inputs from other modalities or additional tasks can have a profound effect on pain perception. Distracting tasks such as algebraic calculation or simultaneously applied pain stimuli lead to a reduction of pain-related activation in specific regions. In this study, pain-evoked cortical activity was monitored during a task specifically activating the sensorimotor part of the brain.

Data were obtained from fourteen healthy male subjects. Electric (EEG) and magnetic (MEG) responses were recorded with pain-inducing stimuli arranged in three blocks (PRE, TASK and POST) of sixty trials each. Radiant heat pulses generated by a Thulium laser were used to deliver stimuli above individual pain threshold to the back of the subject's right hand. Cortical activity was monitored using 64 channel EEG and 31 channel MEG (SQUID gradiometer sensors; array centered over electrode C3). In addition, four electrodes were mounted to the left cheek to record the masseter muscle EMG. Three seconds after each stimulus a tone signal prompted the subject to rate the strength of the pain sensation using a standardized scale between zero (no sensation), four (beginning painful sensation) and eight (strong pain, highly unpleasant). During the TASK block, EMG amplitude was used to generate a frequency modulated audio signal which was fed back to the subject. Using this signal, subjects had to minimize muscular tension. During the PRE and POST blocks, no additional task was provided.

During all blocks, event-related signals peaked around 170ms and 290ms after pain-inducing stimuli. Using a single moving dipole and a spherical volume conductor, localization was accomplished for MEG and EEG recordings. For the first activation peak, MEG data permitted the identification of a source in the contralateral secondary somatosensory cortex (SII). Based on the EEG data, the second peak at 290ms could be attributed to a source in the posterior part of the cingulate gyrus (GC). During the TASK block, mean amplitudes for both MEG and EEG responses decreased significantly (50% and 60%, respectively). Pain ratings showed a reduction of the perceived pain strength during the TASK block.

The pain-related activation observed here is in good agreement with previous studies. The SII response peaking at 170ms is commonly assumed to reflect the discriminative part of pain processing mediated by the lateral pain system. The second response component peaking at 290ms is taken to indicate the aversive emotional processing of pain, carried by the medial pain system located in the GC. The latter also acts as an integrator for signals from other brain regions. Previous studies employing an algebraic distraction task observed an activity reduction mainly in the GC. In contrast, our data show a reduction in both SII and GC activation during the sensorimotor task. This effect might be due to either inhibitory modulation at a low (probably thalamic) level or, alternatively, to local inhibition within the sensorimotor cortex resulting in a reduced forward input to GC.

244 Three-dimensional neurochemical architecture of a novel mechanosensory neuropil in the cricket brain

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Insect antennae are best known as olfactory organs. In the cricket, however, our data show that at least 70 % of antennal sensilla are purely mechanosensory hairs of a single type that are 40 µm long and evenly distributed. To accommodate these afferents, the deutocerebrum of the cricket brain contains, in addition to the olfactory antennal lobe and mechanosensory/motor dorsal lobe, a third distinct neuropil, in which primary fibres of these mechanosensory sensilla terminate. This elsewhere unknown neuropil is named the ventral area of flagellar afferents (*vfa*, see also Staudacher E & Schildberger K, *Zoology* 102: 212-226, 1999). We now show the input-output relationships and neurochemical organisation of this neuropil as revealed by axon tracing combined with immunocytochemistry using the confocal microscope and 3D image processing.

Three-dimensional reconstructions following retrograde staining of the antennal nerve reveals the *vfa* as a highly ordered neuropil, in which four main parallel bands of afferents (*I, II, III, IV*) are distinguishable. The major output pathway from *vfa* is formed by 9 descending interneurones (4 with ipsilateral and 5 with contralateral somata). Terminal arborizations of 7 of these cells appear restricted to afferent band *IV*, whereas the 8th and 9th interneurones form a fine, fibre meshwork throughout the whole *vfa* neuropil. Neuronal elements, including many singly identifiable cells, of probable importance for integrating and processing sensory inputs were revealed by combining axon tracing with transmitter immunocytochemistry. Inhibition by local GABAergic interneurones is likely to be a key integrative process in the *vfa*. Fine fibres and varicosities of GABA-immunoreactive interneurones form 4 bands corresponding to the 4 bands of afferents. A particularly dense fibre matrix is concentrated in afferent band *II*. Sensory integration within the *vfa* is likely to be modulated by biogenic amines and nitric oxide. Serotonin and dopamine immunoreactive fibres and varicosities occur throughout the *vfa* and form 4 bands which correspond to the afferent bands; these fibres are particularly dense in band *II*. Histamine- and octopamine-immunoreactive fibres, though present in *vfa*, are more sparse and do not form bands. Histamine-immunoreactive varicosities are lacking in afferent band *I*. Finally, a nitric oxide synthase antibody and NADPH diaphorase histochemistry both revealed putative NO-releasing structures in the same regions of the *vfa*. Putative NO-releasing elements are concentrated within afferent band *I*, and parts of band *III*. All present data will be integrated into a 3D computer-model in order to evaluate the spatial relationships of several different elements simultaneously.

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Functional properties of trichobotria in the bug *Pyrrhocoris apterus*

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Sense and function of sensory hairs in bugs named trichobothria are still poorly understood. Compared among different groups of bugs a reduction of number of individual trichobotria is evident and a reduction in number of sensile types can be measured.

On abdominal segments in adults of Pyrrhocoris alltogether 28 single sensilae can be distinguished. They are arranged in the middle of the first two segments and along the lateral segment border on next segments. Functional tichobotria are also present in the larvae, beginning from the first instar where only trichobotria of group T1 are found in a typical form, instead of T2 or T 3 a bristle like structure or a design of bristle can be seen. In adult animals regarding to the hair shape, relative amplitude of registered signals and functional properties of trichobothria can be classified in three groups named as T1, T2, T3.

Trichobotria of T1 group had always the smallest amplitude of signal, on the other hand the signal amplitude in trichobotria T2 was the biggest, and T 3 exhibited an intermediate size signal. This order was constant, regardles of the absolute value of the potential registrated . Trichobotria T1 and T2 exhibit spontaneus activity without exception. Mean signal frequency for trichobothria of T1 group at ambient conditions was found to be 3420 pulses per min, of T2 236 per min. In trichobothria of T 3 group on the 5th abdominal segment, spontaneous activity was registered only in 5% of all preparations. In spontaneously active sensilae mean frequency of signals of 25 per min was detected. In a series of following experiments we tried to establish the influence of temperature on the activity of trichobothria. Temperature range chosen in these experiments was between 5 to 40°C what roughly corresponded to oscillation of ambient temperature of Pyrrhocoris in the nature. The frequency of spontaneous activity in trichobothria of T1 and T2 group changes with temperature troughout the whole range. Q10 in 5° intervals is decreasing an it was found to be for trichobotrium T1 between 2.9 at high and 1.3 at low temperatures and for T2 between 8.5 and 2.0.

On the other hand, in trichobotrium T 3 the signal frequency first increases with rising temperature and culminates at 25oC. Beyond this temperature the signal frequency starts to drop. Q10 of T 3 group was found to be between 12.2 and 0.8. Trichobothria of T1 group exhibit directional sensitivity. They respond to the deflection with standardised trapezoid stimulus with excitation in one direction and inhibition by deflection in the opposite direction. Trichobothria of T2 and T 3 group respond with excitation to deflection of the hair in any direction from rest position. In stimulated trichobotria we found that in all the phases of the response all three groups exhibit the same temperature depandence of the response to trapezoid stimulus with Q10 between 3 and 1.

246 Action of Metabotropic Group II/III Glutamatergic Blockade in the Nucleus Accumbens Septi in Pigeons in a Visual Discrimination Task

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Previously we have found that N-methyl-D-Aspartic acid (NMDA) glutamatergic receptor glutamatergic in the nucleus accumbens septi (Acc) is concerned with shape discrimination in pigeons. Such function is not affected by either apomorphine (Apo) or lidocaine. In the present work we have studied the action of antagonists of the different types of glutamatergic receptors in trained pigeons. These were bilaterally implanted with cannuli into the Acc. The drug used were CNQX (6-cyano-7-nitroquinoxaline-2,3-dione disodium, a potent AMPA/kainate receptor antagonist, 2.5 μg/1 μl), CPPG (RS-α-Cyclopropyl-4-phosphonophenylglycine, potent group II/III mGlu receptor antagonist, with much less potent antagonist properties at group I receptors, 1 μg/1 μl), and MPPG (RS-α-Methyl-4-phosphonophenylglycine, potent group III/II mGlu receptor antagonist, with higher action at mGlu III, 1 μg/1 μl). No modifications were seen with CNQX (non-NMDA antagonist) and MPPG, but the percent of correct trials was clearly increased ($p < 0.01$) and the correcting trials were decreased by CPPG ($p < 0.01$). All these findings could be considered as a cognitive improvement with the mGlu II metabotropic blockade in this experiment, suggesting a nootropic action of these compounds in this animal model.

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247 Effects of Increasing Doses of Cycloleucine Injected into the Nucleus Accumbens in the Plus Maze Test in Rats

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The Nucleus Accumbens Septi (Acc) has been extensively studied because of its role in several behavioral processes, like locomotion, stereotipies, reward and some cognitive functions such as learning, memory and visual discrimination. The glutamatergic transmission of the Acc has been recently involved as a relevant neural substrate in the modulation of anxiety. In previous studies we have observed that the blockade of N-Methyl-D-Aspartic Acid (NMDA) receptors led to decreases in anxiety levels measured in rats in the plus maze test. The aim of the present work is to study the action of several doses of Cycloleucine (1-Aminocyclopentanecarboxilic acid), a NMDA receptor antagonist acting at the allosteric glycine site, in male rats bilaterally cannulated into the nucleus accumbens (Acc). Rats were divided into 5 groups (n=19-26) that received either 1 μl injections of saline, or Dizocilpine (0.5, 1.0, 2.0 or 4.0 μg). Time spent into

the open arm was significantly increased by 0.5 ($p < 0.01$), 1.0, 2.0 ($p < 0.05$) and 4.0 μg ($p < 0.01$) when compared with saline controls. Present results show that Cycloleucine has an anxiolytic-like effect when injected into the Acc. We conclude that this pharmacological procedure leads to behavioral disinhibition of cortical pathways with all doses here used.

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Effects of Increasing Doses of Dizocilpine Injected into the Nucleus Accumbens in the Plus Maze Test in Rats 248

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The Nucleus Accumbens Septi (Acc) has been extensively studied because of its role in several behavioral processes, like locomotion, stereotipies, reward and some cognitive functions such as learning, memory and visual discrimination. The glutamatergic transmission of the Acc has been recently involved as a relevant neural substrate in the modulation of anxiety. In previous studies we have observed that the blockade of N-Methyl-D-Aspartic Acid (NMDA) receptors led to decreases in anxiety levels measured in rats in the plus maze test. The aim of the present work is to study the action of several doses of Dizocilpine (MK-801, (5S,10R)-(+)-5-methyl-10,11-dihydro-5H-dibenzo(a,d)-cyclohepten-5,10-imine maleate) in male rats bilaterally cannulated into the nucleus accumbens (Acc). Rats were divided into 5 groups (n=19-26) that received either 1 μl injections of saline, or Dizocilpine (0.5, 1.0, 2.0 or 4.0 μg). Time spent into the open arm was significantly increased by 2.0 ($p<0.05$) and 4.0 μg ($p<0.01$) when compared with saline controls. Present results show that Dizocilpine has an anxiolytic-like effect when injected into the Acc. We conclude that this pharmacological procedure leads to behavioral disinhibition of cortical pathways with the higher doses here used.

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Effects of Selective Glutamatergic Ionotropic Blockades in the Nucleus Accumbens in a Working Memory Test 249

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Effects of blocking N-methyl-D-aspartic acid (NMDA) and non-NMDA glutamatergic receptors on performance and search strategies in the hole board test were studied in male rats bilaterally cannulated into the nucleus accumbens (Acc). Rats were divided into 5 groups (n=17-26) that received either 1 μl injections of saline, (+) 2-amino-7-phosphonoheptanoic acid (AP-7, 0.5 or 1 μg) or 2,3 dioxo-6-nitro-1,2,3,4,tetrahydrobenzo-(f) quinoxaline-7-sulphonamide disodium (NBQX, 0.5 or 1 μg)

10 min before testing. Ambulatory movements were increased by AP-7 (0.5 μg; $p < 0.05$), like non-ambulatory movements and number of movements (1 μg; $p < 0.05$). Sniffing and total exploration were also increased by AP-7 (1 μg; $p < 0.01$). When holes were considered in order (from the first to the fifth by the number of exploratory behaviors displayed into them by each rat), exploration into the most visited holes of the AP-7 group (first and second holes) were significantly higher than the corresponding holes of saline group ($p < 0.05$ for 0.5 μg, and $p < 0.001$ for 1 μg). When the second hole was compared with the first of his group, a difference was only observed in the AP-7 1 μg group ($p < 0.001$). Increasing differences between the other holes and the first were observed by drug treatment. We conclude that NMDA-glutamatergic blockade in the Acc leads to stereotyped, perseverative behavioral disinhibition of cortical influences, related to goal directed behaviors.

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250 Presynaptic afferent depolarization in crayfish mechanosensory afferents is modulated by nitric oxide.

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In crayfish imposed movements of the terminal appendages, the uropods, cause deflection and activation of sensory hairs located on their surface. The actual movements of the uropods are monitored by proprioceptive chordotonal organs within the tail fan whose activation leads to inhibitory inputs in sensory hair afferents. This presynaptic inhibition thus controls the effectiveness of the exteroceptive sensory channel over long time periods. Here we investigate whether inhibitory inputs, elicited in response to stimulation of the protopodite-endopodite chordotonal organ (PEnCO) are subject to modulation by the gaseous neuromodulator nitric oxide (NO).

Inhibitory inputs, in the form of primary afferent depolarizations (PADs), were recorded intracellularly from sensory hair afferents and elicited by mechanical stimulation of PEnCO. NO levels were altered by bath-application of the NO-precursor L-arginine, the NOS-inhibitor L-NAME, the NO donor SNAP and the NO scavanger PTIO, respectively, while changes in PAD amplitude were measured. Application of L-arginine or SNAP consistently resulted in a decrease in PAD amplitude, whereas L-NAME and PTIO induced increases in PAD amplitude.

The results suggest that NO may decrease inhibitory inputs to exteroceptive neurons thus enhancing transmitter release at their output synapses. NO is thought to be generated in response to stimulation of sensory hairs and NO mediated decreases of PAD amplitude may result in enhanced excitation of second order neurons over longer periods of uropod movement.

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Can cockroaches detect electrostatic fields? 251

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Cockroaches detect air movements caused by approaching predators using highly sensitive filiform hairs on the paired terminal appendages, the cerci. These same hairs are deflected by static charge, raising the possibility that electrostatic fields can influence the behaviour of cockroaches. Using a Y-pathway choice apparatus, with a small charged polytetrafluoroethylene (PTFE) plate on one arm, we analysed the choice of walking pathways taken by cockroaches.

Control experiments showed that during walking 97% of cockroaches avoided the charged arm of the choice apparatus (n=35). To understand how cockroaches detect electric fields we analysed the influence of a number of sensory structures during walking. We ablated different combinations of the cerci, antennae and palps from first and second instar cockroaches. These systems were chosen as they all showed movement when exposed to charge. For example, the filiform hairs on the cerci, and the antennae were deflected by electric fields. When cockroaches with ablated cerci were tested in choice experiments, 97% still avoided the charge (n=35), indicating that cerci alone contribute little to the initial sensing of electrical fields. Further removal of sensory systems resulted in more errors. With no antennae, but intact cerci, 24% approached the charge (n=41); with antennae and cerci ablated, 41% approached the charge (n=34), and with palps, antennae and cerci ablated, 75% approached the charge.

We conclude that the cerci alone do not contribute to the detection of electric fields but in combination with more anterior sensory systems lead to the avoidance of electric charges.

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Innervation, distribution and central projections of the paraproctal sense organs and their role during oviposition and mating behaviors im the female desert locust (*Schistocerca gregaria*) 252

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The hypothalamic suprachiasmatic nucleus (SCN) is the primary circadian pacemaker (biological clock) in mammals that controls various rhythms of behaviour, metabolism and physiology. These rhythms with a period length of ~24 hours are synchronized with the external light/dark cycle by retinal photoreception and transmission of light information via the retinohypothalamic tract (RHT). Besides this direct connection with the retina, numerous other brain regions project to the SCN and relay information about the environmental state to the nucleus. This information is integrated by SCN neurons and transmitted to different neuronal targets: endocrine neurons and autonomic neurons in

the hypothalamus, as well as to areas outside of the hypothalamus. The role of the SCN in adjusting the activity of target neurons with the clock signal is not clear at present.

In the present study we have developed a technique using multi-microelectrode arrays (MEA) to study in long-duration recordings simultaneously the electrical activity of SCN neurons and target neurons in explants of the hypothalamus of the mouse. Extracellular recordings from acute hypothalamic slices and from organotypic slices cultured on multi-microelectrode arrays mainly exhibited multi-unit activity, mostly without the possibility to discriminate single unit activity. The multi-unit activity was clearly, and reversibly, reduced or completely inhibited by the application of GABA (100 μm) or by tetrodotoxin (TTX, 1 μm). Neurons within the mouse SCN and within regions adjacent to the SCN, including the paraventricular nucleus of the hypothalamus (PVN), expressed circadian rhythms in spontaneous firing rate with periods of 23.5 to 24.5 hours. The circadian rhythm in hypothalamic areas outside of the SCN disappeared after removal of the nucleus showing that the rhythm is generated by SCN neurons. In acute slice preparations the mean activity showed a peak near midday, at CT 7.0 (CT = circadian time), whereas in organotypic slice cultures the time of peak activity was considerably shifted, due to the absence of a retinal input and the lack of a synchronizing stimulus that is able to adjust the rhythm. The time of peak activity was stable across several cycles in both preparations. Treatment of SCN slice preparations at CT 6 with pituitary adenylate cyclase-activating polypeptide (PACAP, 100 nM), one of the principal neurotransmitters of the RHT, caused phase-advances of the rhythm by 2 to 3 hours; treatment with the pineal hormone melatonin (1 nM) at CT 10 elicited phase advances of 4 hours. However, simultaneous application of PACAP and melatonin at CT 10 failed to exert any effect on the circadian phase of the spontaneous firing rate showing a mutual interaction between both phase-shifting agents.

253 How does the precision of the ant's odometer depend on the distances travelled?

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Foraging desert ants, *Cataglyphis fortis*, monitor their position relative to the nest by path integration (vector navigation). They continually update home direction and distance by employing a celestial compass and an odometer. While the mechanism for estimating direction has been studied in quite some detail (Müller M and Wehner R 1988 Proc. Natl. Acad. Sci. USA 85: 5287-5290), the mode of operation of the ant's odometer is still largely unknown.

The present study addresses the question of how the precision of the odometer depends on the distances travelled. We investigated this question by using linear training channels comprising a nest entrance and a feeder. Ants were trained to forage at seven different distances between 0.5 and 50m. For testing they were caught at the feeder and transferred to a parallel channel where the positions of all U-turns during the nest search were recorded for five minutes. Ants trained to forage at distances > 20m undershot regarding both the position of the first turn and the centre of search. Moreover, the

precision of the odometer decreased exponentially with training distance. The relationship between odometer reading O and travel distance x can be described by $O(x) = I/a_0 \cdot (1 - e^{-(a0/a1)\cdot x})$ where I is the input to the odometer per unit length travelled, e.g. the length of an ant's stride. It represents the solution of the first-order linear differential equation that describes the characteristics of a leaky integrator. Such a mechanism for ant odometry can potentially account for the observed exponential decrease of precision with distance covered, and it offers a plausible explanation for the ant's tendency to underestimate home distance. In natural environments turning before the home vector has been played out leads to the major part of the nest search taking place within an area with landmarks that are familiar to the ant. Hence, from a functional point of view undershooting could be adaptive.

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Self-adapting model-based motion capture system for the analysis of insect movements

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Quantitative analyses of insect limb movements require time consuming manual inspection of recorded videos or a robust motion capture system, which allows automatic limb tracking.

Common motion capture systems use several high speed cameras to identify markers and resolve occlusions and ambiguities. Although powerful, commercial systems are generally not suitable for the analysis of insect motion, because of temporal and spatial constraints of the experiments. One or two views and a PAL video time resolution of 20ms (50Hz) is too low to record smooth 3D marker trajectories, which means that point tracking algorithms are not sufficient. Existing systems use knowledge about marker trajectories to perform a kinematic reconstruction of the observed body movements, while our solution uses a kinematic model of the insect to infer the most likely limb posture.

The limbs of an insect are marked with retroreflective markers, and movements are filmed using one or two standard CCD-camera views. Image processing functions (e.g. thresholding, erosion filters) are used to separate marker pixels from body or background areas in the AVI video files. A 3D kinematic model of the insect morphology and the attached markers is manipulated such that its projection onto the view plane fits the observed marker configuration in each frame. The search for the correct posture of the skeleton model can be expressed as a non-linear optimisation problem, where joint angles are varied to minimise an error function. The error function is the sum of Euclidean distances between markers in the video frame and the markers of the model in all view planes. Additional views of the scene can increase the accuracy of the error function, making the system more reliable. However, the search space is intractable without additional constraints. These are given by the physical joint angle ranges and a set of likelihood distributions, which express the natural variability of the movement parame-

ters. A search algorithm based on the simulated annealing [1] approach exploits this knowledge to search only the most likely joint configuration sub-space. Simulated annealing is a Monte Carlo method, which converges into the global minimum of the error function while avoiding local minima. The resulting joint configurations adapt the likelihood distributions online, improving the performance of the algorithm with each analysed sequence.

The system has been used to analyse locust leg movements recorded using a single camera view. Because of its insect model, the software is able to extract 3D information to some extent even from a single view. It is currently being adapted to work on two or more views of both stick insect and locust motion. Exploiting knowledge about morphology and movement types makes it robust against marker occlusions or errors (e.g. reflections from the shiny wing cuticle). The presented low cost motion capture system is running on a standard PC and is reliable in real experimental situations when using only one or two standard cameras. It is self-adapting, increasing its performance in every run. The adapted likelihood distributions also contain high-level information about the observed movement.

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255 Efficient movement strategies for insect antennae: A modelling study on active tactile sensors

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Stick insects and crickets continuously move their feelers during walking. Although many insect species share basic 'construction principles' of their antennae, such as three functional segments connected by two hinge joints, the joint axis orientation and particularly the movement strategies vary considerably between species. Active movement allows a nearly one dimensional sensor to sample a 3D-volume. However, because the sampling rate of an active tactile sensor is low compared to vision or audition, an appropriate choice of morphology and movement strategy is required to maximise the sampling efficiency for a given environment. Here we undertake a modelling study of active tactile feelers to quantify the effects of various construction principles on the sampling efficiency, to understand species differences and to promote the use of active tactile sensors for biomimetic robotics.

Three attempts were undertaken to clarify the different effects of morphology and movement patterns on sampling efficiency. First, the impact of morphology on the workspace of the antennae was analysed. The reachable workspace of an antennae has a torus-like shape. An appropriate choice of axis angles optimises the torus shape and increases the sampling accuracy by reducing the torus surface to the regions of main interest. Slanting the flagellum relative to the second axis results in an asymmetric torus that narrows the 'out of reach' zone on one side while widening it on the other. Two

symmetric antennae can thereby improve their common workspace in terms of accuracy, region of interest and overlap.

In a second step, we used the morphology of the stick insect *Carausius morosus* and examined the tactile efficiency of various movement strategies. Stick insects perform rhythmic movements with their antennae that are coupled to the stepping cycle of the front legs. This can be modelled by sinusoidal modulation of the joint angles. Sampling efficiency was tested for different combinations of modulation frequencies. A frequency-efficiency plot shows different maxima depending on the structure and orientation of objects in the environment. Even for an unstructured environment the optimal modulation frequencies for the two joints are unequal.

Third, co-evolution of both the morphology and the movement strategy was carried out by means of an evolutionary algorithm in three different types of virtual environment. The objective was to evolve a population of artificial feelers with random construction and movement patterns with the primary goal to maximise the sampling efficiency, i.e. the number of detected obstacles, while reducing energy consumption by keeping the modulation frequencies and amplitudes as low as possible. The first environment consisted of a cloud of uniformly distributed spheres of random size and speed. The second and third environment consisted of horizontally respective vertically aligned cylinders with random speed and diameter. Results show, for example, that winning individuals in the environment with horizontal cylinders moved the proximal joint faster than the distal, while this was reversed in the environment with vertical cylinders. One would expect that changing the environment from horizontal to vertical cylinders would result in rotation of the first axis by 90 degrees, but generally, changing the movement pattern was preferred over changing the morphology by the evolutionary algorithm. A possible reason could be that changing the movement pattern requires less variables to be mutated than changing the morphology.

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Stick insect locomotion in a complex environment: Climbing over large gaps

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When walking in a complex environment, a stick insect may be challenged by various types of obstacles. This requires the controller of the walking system to be highly flexible. Locomotion of stick insects climbing over large gaps has proved to be a useful paradigm to describe how walking behaviour can be adapted to external conditions. In this study, locomotive behaviour of stick insects climbing over gaps of more than twice their step width has been analysed in detail. Special interest has been taken in the question to what extent the sequence of behaviour and the order of its elements (motor primitives) is fixed and to what extent it is variable. The experiments showed that climbing across large gaps requires qualitative and quantitative modifications of the walking controller on different levels. Results lead to the hypothesis that gap crossing behaviour can be regarded an extended type of walking behaviour with exception of an early time

interval of exploration. During this part of the sequence, the animal is not aware of its path to continue and performs extensive exploration movements. The sequence of behavioural elements shows the highest degree of variability during exploration, and the most profound adaptations to different gap sizes also occur in this interval. Initial contact of an antenna or front leg with the second footbridge represents a “point of no return” after which the behaviour is always successfully completed. During the remaining part of the gap crossing sequence, the behaviour is more sequential and includes less “loops”. Modifications of basic mechanisms on the single leg level provide for a high adaptability. These modifications mainly concern spatial and temporal parameters of different step types and the coordination between legs. Adaptations of the walking controller that facilitate slowing down of forward movement, variable searching trajectories of legs in protraction and the generation of short explorative steps are necessary for gap crossing behaviour. The rules of coordination previously described for stick insect walking behaviour (Cruse 1990) also seem to be partly modified, and load seems to play a major role in leg coordination. By implementing these properties into the walking simulation WalkNet (Cruse et al. 1998) we intend to enable the agent to cope with a complex environment in which large gaps can be encountered. Integration of these strategies into the walking controller may also improve our understanding of the ability of insects to adapt their locomotion to highly irregular environments.

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257 Different arm-movement vectors during an eye-hand-task affect the activity of single saccadic neurons in the superior colliculus of a macaque monkey

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In previous experiments we studied the effects of combined eye-hand-movements on the burst-activity of saccade related neurons in the superior colliculus of a macaque monkey. We found a significant modulation - enhancement and reduction of the saccadic burst - in 63 % of the saccadic neurons. The modulation became obvious when comparing the saccadic activity in a purely saccadic task with the activity in a task, where eye-hand-coordination was required. This effect might arise from the activity of reach-neurons with gaze centered frame of reference being active during arm-movements. These neurons lie in direct neighbourhood to the saccadic neurons in the intermediate layers and might interact with them.

To test this hypothesis and to have a closer look at the direction specificity of this effect, a monkey was trained to make saccades into the motion field which was assessed as the visual receptive field in the colliculus‘ superficial layers during the same penetration. Once a single saccadic neuron was isolated the monkey had to perform different paradigms concerning eye- and hand-movements.

We tested the influence of various arm-movment vectors on one single saccade vector. The starting-point of the saccade as well as the endpoint for the eye and hand-movement

was the same in all of the three conditions. In the first condition the hand started at the same location in space as gaze did (ipsilateral hemifield regarding the examined colliculus); different starting-points for the hand-movement were chosen in the second (starting-point in the contralateral hemifield) and third (lower visual field) condition. The three conditions were tested in an interleaved protocol.

Different types of modulation could be found during the experiment. The saccadic bursts as well as the pressaccadic acticity of single cells were affected by the different directions of arm movements in a specific way. Averaging the population of cells measured, these effects perish. This might be due to the fact, that the location of the endpoint is identical for the eye and the arm. For further analysis, paradigms in which eye and arm are being moved by the monkey to different endpoints have to be performed.

Spontaneous modulation of human motor cortex excitability: Noise or rhythm? 258

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Despite much knowledge dealing with activity-dependent modulation in Motor Evoked Potential (MEP) amplitudes to Transcranial Magnetic Stimulation (TMS), little is known about the high variability that MEPs themselves intrinsically display when collected throughout time in resting subjects. Previous studies concluded that such variability likely reflects background noise in the activity of cortico-subcortical neural structures.

Here, the issue of temporal MEPs variability has been reconsidered. This is because either the experimental paradigms and the statistical methods of analysis adopted in the studies referred above may be not well suited to reveal the presence of any periodicity in the MEP time-series investigated. To do this, MEPs have to be collected for a sufficient period of time and care must be taken in order to avoid any behavioural activation throughout recordings. Moreover, parametric methods of analysis (mean, variance) should be avoided for the analysis of time-series which are highly variable in the time-domain, such as the case for MEP amplitudes, since they may understimate the presence of recursive events.

Five healthy young subjects were studied. Each subject underwent five sessions of TMS, each being separated by 24 hours. Complete muscular relaxation was maintained during stimulation. TMS was delivered at an intensity 120% motor threshold by using a circular coil placed over Cz. The coil was maintained in the same position in respect to the site of stimulation by means of an apparatus which allowed an accurate stabilisation. The current in the coil flowed anticlockwise thus allowing preferential stimulation of the left hemisphere and MEPs were collected from the thenar eminence musculature of the right hand. TMS was delivered at a constant rate of 0.2 Hz for 40 min (total 480 stimuli for each recording session). After removal of linear trends, each MEPs time-serie was sub-

mitted to spectral analysis by using the FFT and AR procedures. Whenever present, we considered significant those periodicities which were found to be 2,7 standard deviation above the mean spectral power.

Results demonstrate that MEPs variability is not a stochastic process but has instead an "internal" structure with significant periodicities found in all MEP time-series investigated. The power spectra were weighted towards the low-frequency periodicities with clear power-law scaling behaviour. This pattern is reminiscent of many free-running physiological signals generated by complex, self-regulating, systems displaying 1/f dynamics and fractal, scale-invariant, properties. Nonetheless, when searching for the most repeatable periodicities superimposed upon 1/f-like spectra, significant fluctuations were found at mean periods of 4 min and 20 min with variable repeatability in different subjects.

These periodicities are interpreted as the electrophysiological expression of ultradian fluctuations in cortical-subcortical neural activity, maybe related to concomitant fluctuations in arousal level. The possibility that the same 1/f behaviour in cortical excitability modulation may be imposed by these ultradian fluctuations in cortico-subcortical neural activity is also discussed, together with the possibility that the study of intrinsic cortical excitability modulation may have potential applications in clinical practice.

259 Sensory inputs and the control of aimed leg movements in the locust

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Locusts respond to tactile stimulation of their wings with repetitive scratching movements of their hind legs that are accurately directed towards the site of stimulation. We have sought to understand the role of sensory inputs from the hindwings and legs in controlling this targeting. The likelihood of eliciting a scratch is dependent on the region of the wing being stimulated. This regional sensitivity can be explained by the distribution patterns of three types of sensilla on the hindwing. Basiconic sensilla comprising both chemosensory and mechanosensory afferents are evenly distributed over the wing surface, whereas purely mechanosensory sensilla are restricted to the wing veins. The longest mechanosensory sensilla are present solely along the anal vein (A1), which defines the dorsal fold of each wing, and is the most effective site in eliciting a scratch. The long hairs stand upright, making them ideal for detecting tactile stimulation from above. A sexual dimorphism in the number of mechanosensory sensilla may go someway to explaining sex differences in scratching behaviour.

Ablation of a hindleg proprioceptor (the femoral chordotonal organ) was used to remove a key source of information encoding leg position. Locusts were unable to fully compensate for the absence of proprioceptive feedback caused by the ablation, thus the targeting accuracy of both anterior and posteriorly directed scratches was markedly reduced.

These findings may help us better understand the roles of exteroceptive and proprioceptive sensory inputs, which must be integrated in order to enable accurate targeting.

This work was supported by the BBSRC.

Temperature sensitivity of motor behaviour and its neurophysiological control in marine crustaceans from different thermal environments

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The effect of temperature on the neuronal control of behaviour was assessed for four marine crustacean species from stable or variable thermal environments: *Carcinus maenas* and *Ligia oceanica* from the British coastline, where temperature varies between 2 and 24 deg C annually, and *Glyptonotus antarcticus* and *Paraceradocus gibber* from Antarctica, where the temperature is a constant –1.8 deg C.

The temperate species produced coordinated behaviour up to at least 20 deg C, but the Antarctic species became uncoordinated above 5 deg C and died at approximately 8 deg C.

There were positive linear relationships between temperature and neuronal conduction velocity in the pereopodal (leg) nerves of all four species between 0 and 22 deg C. In contrast, measurement of how these action potentials are converted into post-synaptic potentials at the neuromuscular junction (NMJ) showed that the temperature at which electrical stimulation of the pereopodal nerve failed to elicit potentials in the muscle depended on the temperature tolerance of the species. Intracellular recordings from *C. maenus* showed an exponential decrease in the time constant of decay of excitatory junction potentials (EJPs) between 5 and 10 deg C, with EJP failure as the temperature exceeded 20 deg C. Recordings from *P. gibber* showed similar declines in both facilitation and the time constant of decay of EJPs as temperature increased above 0 deg C, with EJP failure occurring between 8 and 12 deg C. Experiments to determine why *P. gibber* cannot produce EJPs above 8 deg C showed that the resting potential of muscle fibres remained constant or decreased (i.e. became more positive) as the temperature was raised, rather than increased as predicted.

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261 Contribution of the maxillary muscles to proboscis movement in hawkmoths (Lepidoptera: Sphingidae) - an electrophysiological study

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The mechanism of proboscis movement in Lepidoptera is debated controversially since Réaumur (1734). Coiling and uncoiling movements have been variously attributed to intrinsic galeal musculature, hydrostatic pressure change within the galeal lumen, and elastic properties of the proboscis cuticle. Present hypotheses are based on morphological or mechanical studies (e.g. Schmitt 1938, Eastham & Eassa 1955, Bänziger 1971, Krenn 1990, 2000). Thorough physiological studies, however, are lacking to date.

In the present study the contribution of the maxillary muscles to proboscis movement was examined by means of extracellular electrophysiological recordings combined with video analysis. Seven sphingid species with particularly long or stout proboscides were investigated.

The proboscis comprises the galeae, each of which contains two short basal muscles (GBM) and two series of intrinsic oblique muscles - a ventral (GVM) and a lateral (GLM) one. The stipes consists of a tubular and a flat part, with the latter bearing a broad apodeme. Two muscles insert on this apodeme, taking their origin broadly on the anterior tentorial arm (STM) and on the gena (SGM), respectively. A third muscle arises posteriorly on the anterior tentorial arm and inserts at the distal end of the stipes (SPM).

Proboscis extension is initiated by an elevation of the galea base caused by the GBM. The actual uncoiling of the proboscis spiral occurs in several distinct steps, each of which is accompanied by a compression of both stipites. Each compression is caused by a simultaneous contraction of STM and SGM which are assumed to force hemolymph into the galeal lumen. No galeal GVM/GLM or stipital SPM activity is measurable during extension. The measurements confirm the hypothesis that proboscis extension is caused by an increase of hemolymph pressure within the galeae.

Recoiling occurs in two steps. Initially, the proboscis recoils into a loose spiral and since no muscle activity - neither stipital nor galeal - is measurable, this step seems to be due to elastic properties of the galeal cuticle. Completion of coiling into a tight spiral with consecutive coils being brought into close contact to each other is caused by a contraction of both galeal GVM and GLM. Finally, a downward movement of the galea base, caused by the stipital SPM, is observable, which brings the proboscis back to its resting position.

Cooperation and leg motor control of the graviceptive interneuron pair in the cricket CNS

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Gravitation is a force that acts permanently on every animal. Special problems arise in behaviors such as walking, flying or jumping. Nevertheless only very few insects (gryllids, stenopelmatids and some cockroaches) have specialized equilibrium organs. All the others are dependent on calculating the vector of gravitation by the use of proprioreceptors not specialized for this stimulus.

The equilibrium organ of *Gryllus bimaculatus* consists of approximately 150 club-shaped hairs on the proximo-medial side of each cercus. Only one bilateral pair of position-sensitive interneurons (Ψ) integrate these phasic-tonic afferents in the terminal ganglia and project anteriorly through the connectives at least to the prothoracic ganglia. Back and forth tilting results in synchronous changes of spike frequency in both PSIs, where tilting backwards leads to strong frequency increase and forward tilting to a weak decrease. Lateral tilting of the cricket increases heavily the spike frequency of the Ψ projecting through the connective on the side to which it tilts. In the other Ψ the frequency remains however nearly unchanged. From combinations of these responses in two neurons all motor reactions to gravity depend. This bottleneck in transporting spacial information from the terminal ganglia to the motoric centers of the thorax is used to explore the principle of gravitation-inducted motor activity.

Related efferents in the mesothoracic ganglia could be observed in nerve 1A (ventral longitudinal muscle (M60), sternospinal, intersegmental muscle (M59)), 3D1 (anterior rotator of the coxa (M92)) and 3D3 (thoracic part of the depressor trochanteris (M103c)).

Efferents in nerve 3D3 react to tilting to the right as well as to the left with increasing spike frequency. Back and forth tilting leads to no reaction. After the cercal nerves were severed a residual reaction was seen, suggesting another gravitation-sensitive system may exist.

Driving the spike frequency in one of the two PSIs by intracellulare depolarization, pretending inclination, leads to the a reduction of the ipsilateral spike frequency in the N3D3. This contradicts the observations in intact animals. Possibly *Gryllus bimaculatus* has two gravitation-sensitive systems in its body each dominant in special behavioral contexts. One like in all other insects for standing and walking, the 'club-shaped hair-Ψ'-system for jumping and flying.

263 Melatonin: A candidate compound for neuroprotection in amyotrophic lateral sclerosis (ALS)

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Amyotrophic lateral sclerosis (ALS) is a fatal motor neuron disease, affecting both cortical and spinal motor neurons. The progression of ALS is characterized by degeneration of neurons associated with a dramatic demyelinisation in the anterior horn of the spinal cord. The etiology is only partially understood. Therapeutic possibilities are very limited. Any neuroprotective approach, resulting in an improvement of quality of life, reduction of neurological symptoms, and slowing of disease progression will represent a major achievement in this disease. Melatonin has a unique broad spectrum of effects including scavenging of hydroxyl carbonide, alkoxyl, peroxyl, and aryl cation radicals, stimulation of glutathion peroxidase and other protective enzymes as well as suppression of NO synthase. This combination of properties is not provided by any classical antioxidants, but addresses optimally the antioxidative requirements for treatment of ALS.

We have been comprehensively exploring the neuroprotective potential of melatonin in various transgenic models of neurodegeneration, including demyelination and motor neuron disease. Using SODG93A-transgenic mice as an animal model for ALS, we were able to show significant improvement in the course of disease, including several functional and behavioral parameters, e.g. rotarod performance, progression of paresis or weight loss, upon high dose oral melatonin treatment. No adverse effects of systemic melatonin were observed, even after application for several months.

These experiments have stimulated us to perform a first small clinical safety trial introducing long term application of high daily oral dose of melatonin. We found that melatonin is well tolerated and safe in ALS patients. In preparation of a clinical trial on melatonin treatment of ALS, we identified diffusion tensor imaging (DTI) as a sensitive measure of progressive corticospinal tract degeneration in ALS patients with leading upper motor neuron involvement. Complementary to the clinical follow-up, this measure will be used as an objective morphological marker to assess the success of therapeutic trials.

Anticipation of dynamic targets during eye-hand-coordination 264

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To grasp a moving target object requires the prediction of its movement trajectory. It is hypothesized that subjects have the capability to develop a motor schema to anticipate the path of a moving target and to compute an optimal course of interception. During the performance of perception-action-cycles such a schema may highly reduce the control load and thus the question arises, what are the optimization characteristics.

Subjects were asked to hit a moving target circle, 6 mm in diameter, by means of a cursor. The position of the target and the cursor was displayed on a screen. The target moves along a circular path with a constant radius of 12 cm and different velocities. The cursor position was controlled by the help of a graphic tablet. The experimental set-up allows to record eye and head movements in order to compute the gaze position on the screen. After training 12 subjects were analysed in two subsequent sessions. Each experimental session consisted out of 446 parts during which the subject had to perform a single move to hit the target. Every experimental part included the following sequence. Moving the cursor to the marked centre of the circular path made the target circle appear. Moving the cursor towards the target and exceeding a given distance (*lrt* and *lrc*) from the centre made the target and the cursor disappear, respectively. This allows to analyse the effect of anticipating the target movement and the requirements of feedback. Six different target velocities between 0 and 12.3 cm/s were chosen. The distance parameters *lrt* and *lrc* were varied between 6 and 12 cm.

In the beginning of a single experimental part the cursor has to be moved to the marked centre of the circular path of the target in order to make it appear. This allows to compute the latency of the eye and hand movement towards the target. Obviously the randomized initial target position and its velocity is detected in the periphery of the eyes and results in a saccade towards the target in order to fixate it. Even when the target disappears the eyes follow its anticipated course. The latency of the onset of the eye movement is in the range of 370 ms and the latency of the onset of the hand movements is in the range of 470 ms. Before the saccade is initiated position and velocity of the target is computed. Therefore, hand movements start with a lead which is correlated with the velocity of the target. The latencies of eye and hand are hardly affected by the chosen independent parameters. However, the analysis of the error rate showed that a decrease of *lrt* and *lrc* leads to a clear increase in target misses, respectively. The anticipation becomes more and more imprecise in controlling the cursor.

The subjects are able to develop a stable and effective strategy on approaching the target even during early disappearing of target and cursor, respectively. During the start phase, that is the ballistic part of the move, there is a highly significant lead of the hand with respect to the target position and during the final phase the hand movement lags behind. The course of the trajectories clearly depend on target velocity. Therefore, one has to assume that the subjects develop a specific schema which is based on optimizing certain movement parameters. A dynamic model has been developed on the basis of biomecha-

nical considerations to describe the optimal movement trajectories with respect to varying velocities of the target.

265 Mirror movements, mirrored EMG activity and ipsilateral MEPs in ALS patients

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The clinical observation of mirror movements in ALS patients prompted us to investigate mirrored motor activation by bilateral EMG and MEP recording. The intention of our study was to detect discrete signs of upper motor neuron (UMN) involvement in ALS by this approach. 25 patients with possible, probable ore definite ALS, six patients with clinically suspected ALS without detectable signs of UMN involvement, 19 disease controls and five healthy volunteers were included. In five ALS patients (16 %) mirror movements of the resting hand were observed when contralateral sequential finger-thumb opposition was performed. In 17 (55 %) of the ALS patients surface EMG allowed the registration of muscle activity in the contralateral first dorsal interosseous muscle upon sequential finger-thumb opposition in the contralateral hand.

MEPs in response to single shock TMS were bilaterally recorded from the abductor pollicis brevis muscle. M and F waves were elicited by supramaximal stimulation of the median nerve and central motor conduction times (CMCTs) were calculated using the F wave latencies. Ipsilateral MEPs could be obtained in 17 (68 %) out of 25 ALS patients and in three out of six patients with clinically suspected ALS. CMCTs were prolonged in 16 ALS patients (64 %).

The occurence of mirrored EMG activity and ipsilateral MEPs may be an early electrophysiological sign of the involvement of UMN in ALS.

266 Directional Tuning of Motor Cortical Neurons During Continuous and Reaching Movements

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Arm related neurons in the motor cortex are thought to represent the direction of arm movements. This is expressed by the tendency of a single neuron to elevate its firing rate mostly before and during a movement in a certain direction, the preferred direction (PD), and reduce it as the difference between movement direction and PD becomes larger. This result was obtained for reaching movements, but does it hold when other types of movements are performed?

To examine this issue we recorded neural activity from arm related areas in primary motor and pre motor cortices of a monkey, while it alternated between blocks of scribbling and center-out (CO) tasks. During a scribbling trial the monkey moved the

manipulandum until hitting an invisible target, randomly chosen out of 19 hexagonal targets that tiled the workspace. This produced prolonged, continuous trajectories. During a CO trial a reaching movement was made from a central target to one out of six peripheral targets. This task produced straight trajectories.

Directional tuning curves were obtained for cells during reaching movements by fitting the average firing rates to a cosine and von- Mises functions. Tuning curves during scribbling were obtained for tuned cells by taking pieces of the motion, with a constant direction (± 15 degrees) and fit the average activity before and during the motion to the same functions. Often, the PD obtained for scribbling deviated from the PD obtained for CO. This was consistent in different time lags from 250 ms before to 50 ms after start of motion. The effect of tangential velocity was tested by sorting the motion pieces in each direction according to the velocity profiles and comparing the tuning curves between pieces with similar velocity profiles and pieces with different profiles. Preliminary results show that scribbling tuning curves with CO like velocity profiles deviate from CO tuning curve. Therefore we conclude that directional tuning in motor cortical neurons is not invariant, but depends on the motor task. A possible explanation for this discrepancy is that other parameters, like the behavioral set, that are changed when switching between these tasks are represented in the neural activity.

Intersegmental effects of a leg joint receptor on leg motoneurons in the stick insect **267**

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The coordination of adjacent legs in walking stick insects is well described on the behavioral level (Cruse H (1990) TINS 13:15). We are interested in the neural mechanisms of intersegmental coordination of leg movements. In the stick insect *C. morosus* walking movements of a single front leg modulate the activity of leg motoneurons (MNs) of the ipsilateral middle leg (see poster by Ludwar and Büschges). We investigated influences from the femoral-chordotonal-organ (fCO) of a front leg on the activity of ipsilateral middle leg MNs. The fCO measures the position and movement of the femur-tibia joint (Bässler U (1993) Brain Res Rev., 18:207), which flexes in stance and extends in swing phase of the front leg. Thus, fCO signals could be used for mediating phase locked signals for intersegmental coordination.

Movements of the femur-tibia-joint were mimicked by moving the fCO-apodeme with ramp-and-hold stimuli. Stimuli were applied in two different behavioral states: The active state evoked by tactile stimulation is characterized by high background activity in MNs and a high probability of a local assisting reflex in the femur-tibia joint upon fCO stimulation. The inactive state of the resting animal is characterized by low background activity in MNs and a local resistance reflex in the femur-tibia joint upon fCO stimulation. The activity of MNs supplying the three most proximal joints in a middle leg was recorded extracellularly from nerve stumps of the de-afferented and de-efferented mesothoracic ganglion.

1. MNs of the thorax-coxa joint were affected by mimicked flexion movement of the tibia. Mean activity of protractor coxae MNs was reduced to 76 % of control activity

and mean activity of the retractor coxae MNs increased to 160% of control activity. Responses were the same in active and inactive animals.

2. MNs of the coxa-trochanter joint (levator and depressor trochanteris) were not systematically influenced by stimulation of the fCO regardless of the behavioral state.

3. MNs of the femur-tibia joint were affected by stimulation of the fCO in dependence on the animal‘s activity state. Pulling and relaxing the fCO tendon in the inactive animal increased spontaneous spike activity moderately in extensor and flexor tibiae MNs. In activated animals a mimicked flexion moderately increased mean activity in extensor MNs. In contrast, flexor tibiae MNs discharged a vigorous short latency burst of spikes upon a mimicked flexion movement. There was no response upon relaxation of the fCO tendon.

In summary, information about movements of a prothoracic femur-tibia joint is mediated to motor control networks of the ipsilateral mesothoracic leg. Intersegmental effects of the fCO on MNs are specific for each leg joint.

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268 Recruitment of flexor tibiae motoneurons during walking-like movements of the stick insect

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We were interested in the influence of load on the activity of individual leg motoneurons (MNs) in the stick insect (*Baculum extradentatum*). The walking-like movements of a single middle leg preparation on a treadwheel (Fischer et al. 2001, *J Neurophys* 85, 341-353) were investigated at different levels of belt friction, which is equivalent to a change in load. Intracellular recordings were performed from MNs innervating the flexor tibiae, which is the most important stance phase muscle in this preparation.

At all frictional levels investigated (1.5 to 6.5mN; equivalent to 14-60% of the animal’s weight) we could observe a correlation between belt friction and the force applied to the belt by the animal as well as the flexor EMG activity. The increase in EMG activity at higher levels of belt friction was due to an increase in firing rate of both slow and fast MNs.

Throughout stance phase, the membrane potential of both fast and slow MNs of the flexor tibiae depolarized with a very similar time course. No differences were observed in the activation pattern of the MNs. However, the slow and fast MNs were recruited progressively during stance phase. Slow MNs were active earlier than fast MNs; the half-maximal spike frequency was reached after 10-15% (N=13) and 50-55% (N=10) of stance phase, respectively. The resting membrane potential was more hyperpolarized in fast MNs than in slow MNs (slow: -52.9±5.4mV, fast: 64.6±6.5mV; mean ± SD). In contrast, the threshold for the initiation of action potentials was not significantly different in both types of flexor MNs (slow: -46.8±5.2mV; fast: -50.8±5.3mV). Therefore, action potentials in fast MNs were generated after a longer period of depolarization and thus later during stance phase than in slow MNs.

We suggest that that the difference in resting membrane potential between slow and fast MNs plays a crucial role in their consecutive recruitment as a response to substantial common excitatory inputs.

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Behavioral and Ventilatory Reactions to Illumination in Free Moving Crayfish, *Procambarus cubensis* 269

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The crayfish is known to possess retinal and extraretinal (located in the terminal abdominal ganglion) photoreceptors as well as light sensitive neurons inside the cerebral ganglion. It is obvious that visible light can elicit a broad spectrum of behavioral reactions in the crayfish. In the experiments on free moving crayfish *Procambarus cubensis* grown up in the laboratory, its background locomotory activity was recorded by a non-invasive optical technique. The number of passages along the experimental chamber measured just after placing an animal into the chamber was maximal at the first five minutes; it declined gradually during 15 min. This index was higher for the light-adapted (40-100 Lx) than for the dark-adapted crayfish. Short (30 sec) illumination of dark-adapted crayfish elicited a decrease in the interval between the passages whereas switching light off in the light-adapted crayfish resulted in an increase of this interval. No significant changes in the inter-movement intervals were observed for the 1 s and 10 s light stimuli of the same intensity. The behavioral data are coincident with those of the experiments measuring the ventilatory activity of free moving *P. cubensis*. The reaction to illumination of dark-adapted crayfish consisted in an initial decrease and gradually developing increase of the rate of the ventilatory activity. It is concluded that visible light of moderate intensity has a short inhibitory and long-lasting stimulating effect on different functional systems of the crayfish mediated probably by its cerebral ganglia.

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Eye-head coordination: Challenging the system by increasing head inertia 271

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Under natural conditions, coordinated movements of eye and head are used to shift gaze towards new visual targets. In humans, combined eye-head saccades bring gaze to the target by a fast saccadic eye movement together with a slower head movement. After gaze reaches the target, it is stabilized at the target position by the vestibulo-ocular reflex. The interaction of eye and head during the gaze shift is far from clear. To further

disclose this interaction, we investigated how gaze shifts are affected by changes in one of their components - the head movement.

The head moment of inertia of human subjects was increased using a helmet with a mass attached to it. Eye and head positions of the subjects were recorded during horizontal gaze shifts towards shortly flashed targets in the complete dark without and with loaded head. The primary gaze shift towards the target was analysed.

The increased head moment of inertia significantly slowed down the head and decreased the head amplitude at the end of the gaze shift. These changes in head movement did not affect the retinal error at the end of the gaze shift. Observed changes in eye velocity could be explained by a respective change in amplitude, i.e., regardless of head load eye movements followed the main sequence for combined eye-head movements.

These effects are not due to visual feedback which was excluded by flashing the target. Thus, in order to bring gaze equally close to the target despite of the lower head amplitude, other feedback mechanisms, vestibular or proprioceptive, must be at work. We conclude that the eye-head system is robust against changes in head inertia.

272 Twitch and non-twitch motoneurons of extraocular muscles have different histochemical properties.

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The extraocular muscles of vertebrates show a highly complicated organisation. Their muscle fibres can be classified into two main categories: singly innervated "twitch"-fibres (TMF), resembling those of skeletal muscle fibres and multiply innervated "non-twitch"-fibres (non-TMF). The motoneurons of the extraocular muscles lie in three brainstem nuclei: the oculomotor nucleus, the trochlear nucleus and the abducens nucleus. Recent work in the macaque monkey showed that the motoneurons innervating non-TMFs are located in separate subgroups, which lie around the borders of the classical nuclei (Büttner-Ennever et al., 2001, J. Comp.Neurol.438:318-335). In the present study we investigated, with combined tract-tracing and immunofluorescence methods whether TMF- and non-TMF motoneurons differ in their histochemical properties.

In the macaque monkeys the non-TMF motoneurons in the abducens nucleus were retrogradely labelled after injections of cholera toxin subunit B close to the distal myotendinous junction of the lateral rectus muscle. Free floating brain sections were first processed for the tracer detection by immunofluorescence, and then treated with a second immunoflourescence protocol for one of the following antibodies: anti-chondroitin-sulfate-proteoglycans (CSPG) as a label for perineuronal nets, SMI 32 for non-phosphorylated neurofilaments, anti-parvalbumin (PV) and anti-cytochromoxidase (CO).

The double-labelling experiments revealed that the retrogradely labelled non-TMF-motoneurons in the periphery of the abducens nucleus do not contain PV- and SMI32-immunoreactivity, and they lack CSPG-positive perineuronal nets. In contrast, the TMF motoneurons within the abducens nucleus are PV- and SMI32-positive and they are

ensheathed by prominent perineuronal nets. CO-immunoreactivity was more or less present in both, TMF- and non-TMF-motoneurons.

These data provide first evidence that TMF and non-TMF-motoneurons differ in their histochemical properties. The "negative" markers PV, SMI32 and CSPG may help to identify the homologue non-TMF-motoneurons in other species, including human.

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Do saccades to stationary targets differ from those to moving targets? 273

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Classical models for the generation of saccades use the retinal error (difference of target position and eye position) as the main driving stimulus. If the target was moving and the retinal error would be sampled shortly before saccade onset (dead interval of about 80 ms) such a saccade would be systematically to small to catch an onward moving target. Recently, Optican and Quaia (2001) published a theoretical model that suggests that the additional neuronal input from cerebellum (oculomotor vermis, fastigial nucleus) is used to alter the deceleration phase of saccades to moving targets in order to make them exact. We found experimental support for this hypothesis by studying the saccades of two Rhesus monkeys using a step remp paradigm: after an initial step targets the animals had to look at remained in their position (step) or moved with constant velocity (step - ramp) onward or backward. The analysis of our data led to the conclusions that 1) onward and backward saccades are on different main sequences: onward saccades have a longer duration but a lower maximum velocitiy, 2) latency enhancement increases the amplitude of onward saccades, but decreases the amplitude of backward saccades, 3) saccades to moving targets are not (exclusively) determined by the retinal error but (also) alter their velocity profile due to additional factors.

274 Comparison between monophasic and biphasic transcranial magnetic stimulation of the human motor cortex

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Objectives: To compare motor evoked potentials (MEPs) to monophasic transcranial magnetic stimulations (TMS) of the human motor cortex with those to biphasic stimulation.

Methods: Subjects were 11 healthy volunteers. Surface electromyographic responses were recorded from the first dorsal interosseous muscle (FDI) in every experiment. Several different stimulation experiments were performed in all subjects. In each experiment, suprathreshold consecutive 20 stimuli were given, and the stimulus intensity was fixed at slightly above the resting motor threshold. In motor cortical stimulation we gave both monophasic and biphasic TMS, and 0.5, 1, 2 and 3 Hz frequency stimuli. To study the spinal excitability changes, we also performed 2 Hz TMS at the foramen magnum level (Ugawa et al. 1994). We compared response size and latency changes in MEPs to 20 consecutive stimuli among different stimulation conditions.

Results: The threshold for motor cortical stimulation was significantly lower in biphasic stimulation than in monophasic stimulation. In monophasic stimulation, MEPs to 2 and 3 Hz stimuli gradually increased in amplitudes during stimulation, and the later MEPs were significantly larger than the earlier ones. In contrast, in biphasic stimulation, the size of MEPs did not show significant changes throughout 20 successive stimuli. 2 Hz foramen magnum level stimulation elicited fairly constant MEPs during stimulation. Latencies were almost the same under any stimulation conditions.

Conclusion: The finding that MEPs were enhanced at the late period of 20 stimuli whereas responses to foramen magnum level stimulation were unaffected suggests that enhancement should occur at the motor cortex during repetitive monophasic 2 or 3 Hz stimulation. We suppose that monophasic TMS is more powerful than biphasic TMS in a short-term effects during repetitive stimulation because monophasic pulses preferentially activate one kind of neurons oriented towards the same direction and stimulation effects summate during stimulation, whereas biphasic pulses activate several kinds of neurons (facilitatory and inhibitory) and summation of the same effects should not occur so much. On the other hand, in single pulse stimulation, biphasic TMS is more powerful than monophasic TMS probably because the pulse duration is longer and pulse amplitude is higher in biphasic pulses than in monophasic pulses.

Effects of transient transcranial direct currents over the human hand motor area

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Purpose: Transcranial direct current (tDC) for longer than 5 minutes has recently been reported to elicit a lasting after-effects on the human brain. Anodal stimuli enhance the excitability while conversely cathodal stimuli suppress it (Nitsche and Paulus, J. Physiol 2000: 527; Baudewig et al. Magn Res Med 2001: 45; Nitsche and Paulus, Neurology 2001: 57). The aim of the present communication is to see whether a transient tDC has some influence on the human brain.

Methods: Six normal volunteers participated in this experiment. Electromyographic (EMG) responses were recorded from the right first dorsal interosseous muscle. We gave two kinds of stimulus; tDC (conditioning stimulus (CS)) and transcranial magnetic stimulation (TMS) over the motor cortex (test stimulus (TS)). We used a randomized conditioning-test design in recording, and evaluated effects of tDC on responses to the same TMS. 100ms tDC was given with electrodes over the left M1 and the forehead. Several kinds of tDC were used: motor cortical anodal or cathodal; intensity 1, 3 or 5 mA. As a test stimulus (TS), TMS was given over the left motor cortex. Interstimulus intervals (ISIs) were 0-7 and 10-120 ms. We estimated the effect of tDC by comparing response sizes between the control (TS given alone) and conditioned (CS + TS) responses.

Results: At short ISIs (0-7 ms), cathodal tDC significantly reduced the size of responses to motor cortical stimulation, whereas anodal tDC did not affect them. At longer ISIs, both anodal and cathodal tDC decreased responses to TMS significantly at ISIs of 30-60 ms and increased them at an ISI of 90 ms.

Discussion: Suppression of MEPs at short ISIs suggests that cathodal tDC transiently decrease the excitability of the motor cortex. This is consistent with the results by Paulus et al. Even very small tDC current flowing at the target neurons (about 1/10000 of the current induced by TMS or 1/100 of the currents in the animal experiment (Bindman et al. J Physiol 1964: 172)) can transiently change their excitability at short intervals. Similar phenomenon may explain a long lasting effect of tDC reported previously.

Suppression evoked by both polarity stimulation at ISIs of 30 to 60 ms is akin to the effect produced by trigeminal stimulation, pain or large sound (Siebner et al. Clin Neurophysiol 1999: 110; Valeriani et al. Clin Neurophysiol 1999: 110; Furubayashi et al. Clin Neurophysiol 2000: 111). The effect at later ISIs should be a kind of secondary effects elicited by a week pain or phosphene associated with tDC.

276 A sensory neuron in a positive feedback loop and its influence on a central pattern generator

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Sensory feedback plays an essential role in shaping the motor output produced by central pattern generators. In the stomatogastric nervous system of the crab *Cancer pagurus* two central pattern generators control the movement of the food throughout the foregut: The pyloric network drives the filtering of food and the gastric mill network coordinates the chewing of food. Both networks are strongly influenced by sensory feedback (Katz & Harris-Warrick, 1989, J.Neurophysiol. 62:571-581).

The gastric mill motoneuron DG receives excitation from GPR2, a sensory neuron that innervates the gastric muscle GM9. GPR2 responds to passive muscle stretch (Katz et al., 1989, J.Neurophysiol. 62:558-570), which may be exerted by contractions of the GM4 muscles attached to the base of GM9. *In vitro*, GPR2 also responds to active GM9 contractions elicited by the burst activity of the gastro-pyloric motoneuron MG.

DG and MG usually show alternating activity in a gastric mill rhythm. Because activity in both neurons might activate GPR2 (either by passive stretch or active contraction of GM9), we asked what mechanisms activate GPR2 in an intact stomach. Since GPR2 uses Serotonin as a transmitter which in turn causes plateau potentials in DG (Katz & Harris-Warrick, 1989, J.Neurophysiol. 62:571-581), we also asked how GPR2 affects the generation of the gastric mill rhythm.

In reduced preparations with only the GM4 and GM9 muscles intact, GPR2 fired either in bursting or in spiking mode. In bursting mode, GPR2 spontaneously produced bursts of action potentials even without GM9 muscle stretch or contraction. Each burst strongly influenced the gastric mill rhythm by prolonging the DG bursts in an ongoing gastric mill rhythm. GPR2 bursts were entrained by DG activity. In spiking mode, GPR2 fired tonically at low firing frequencies, but did not produce bursts. DG burst activity was not sufficient to activate GPR2, whereas 20Hz stimulation of DG caused GPR2 to increase its firing rate.

In semi-intact preparations with all extrinsic muscles intact, GPR2 was never found to be in bursting mode. It was either silent or in spiking mode. GPR2 was only activated by muscle movement during a gastric mill rhythm, passive stretch of the GM9 muscle or electrical stimulation of the *dgn* nerve (which contains the DG axon).

GPR2 thus seems to enhance DG activity which in turn activates GPR2 by passive stretch of GM4. Currently, we are testing the consequences of this feedforward loop in an artificially closed loop system.

Does gravity deprivation modify the development of the *Xenopus laevis* vestibuloocular and spinal motor system in a correlated manner? 277

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In *Xenopus laevis*, the static vestibuloocular reflex (rVOR) and the rhythmic activity recorded from the ventral roots (VR) during fictive swimming undergo clear developmental time courses. During the early period of life up to stage 47 (two weeks after fertilization), they revealed a steady increase or decrease of characteristic parameters such as rVOR amplitude and gain (Horn et al. 1986, J. Comp. Physiol. 159A:869) or VR-burst and VR-episode duration. We wanted to find out whether these changes are related to each other because it is very likely that both systems are linked to the vestibular nuclei. - METHODS: Modification of the adequate environmental conditions such as sensory deprivation during early periods of life is a well-known possibility to affect the development of sensory systems; in some instances, this treatment causes long-lasting morphological and/or physiological effects. During the Soyuz taxi flight Andromède to the International Space Station ISS (launch: October 21, 2001; landing: October 31, 2002), we exposed *Xenopus* embryos (stage 25-28) which had not yet developed their rVOR for 9.5 days to microgravity (0g). Former studies have revealed that this period of gravity deprivation is sufficient to weaken the rVOR sensitivity for several days (Sebastian et al. 1996, Exp. Brain Res. 112:213; Sebastian and Horn 1998, Neurosci. Letters 253:171). After the mission, each tadpole was tested for its rVOR and thereafter for its VR-activity. From each sine-like rVOR characteristic we determined the rVOR amplitude and correlated it with the results obtained from the recordings of the rhythmic spinal VR activity patterns of the respective tadpole. - RESULTS: We have found significant correlations between the extent of the rVOR and VR activity either only in 0g-exposed animals (burst duration and rostrocaudal delay) or only in 1g-tadpoles (episode duration). In particular, the rVOR amplitude was positively and significantly correlated with the VR-burst duration and rostrocaudal delay in tadpoles with 0g-experience but not in their 1g-controls. On the other hand, the rVOR amplitude was negatively and significantly correlated with VR-episode duration in the 1g-controls while no correlation occurred in the 0g-tadpoles. - CONCLUSIONS: The development of the rVOR network and spinal motor system reveales features which might be based on an intrinsic overall-relation or on a dependency of these subsystems mediated by descending pathways from the brainstem to the spinal cord.

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278 The effect of altered gravity on the locomotor pattern during the early development of tadpoles (*Xenopus laevis*)

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Tadpole swimming, a stereotyped rhythmical activity, is an excellent model to examine effects of altered gravity on the motor system since the movement simplicity makes it easy to detect basic changes. It is known that freely moving tadpoles (*Xenopus laevis*) show a significant lower tailbeat frequency than 1g-controls, if they had developed under space conditions (Fejtek et al. 1998, J. Exp. Biol. 201, 1917). The swimming pattern is generated by a central oscillator. It is possible to observe the rhythmical, burstlike activity of motoneurons by extracellular recordings from ventral roots of the spinal cord in paralyzed animals (*fictive swimming*; Kahn and Roberts 1982, J. Exp. Biol. 99, 185). - METHODS: Experiments were performed with *Xenopus* tadpoles exposed for 9 to 11 days either to hypergravity (3g) on ground in a centrifuge or to microgravity (0g) on the International Space Station (Soyuz taxi-flight Andromède 2001). Ventral root recordings started 4 hrs after the end of hypergravity and 48 hrs after the end of microgravity exposure. Fictive swimming was induced by a mechanical stimulus. From these recordings, four parameters were examined: the duration of the fictive swimming activity, the duration of the bursts in the 10^{th} segment, the cycle length and the rostrocaudal delay of ipsilateral burst activity between segment 10 and 14. - RESULTS: Hypergravity exposure led to a significant increase of burst duration. The effect was stronger in animals that have been exposed to 3g at the beginning of neurulation ($p<0.01$; mean values 3g: 21ms and 1g: 16ms) than in tadpoles that already produced motoneuron activity ($p<0.1$; mean values 3g: 23ms and 1g: 19ms) at the start of hypergravity. This can be an evidence for a decrease of neuronal plasticity in the locomotor system with increasing age. After 0g-exposure the duration of fictive swimming episodes was significantly increased ($p=0.05$; mean values 0g: 3.0s and 1g: 2.2s) while the rostrocaudal delay was significantly decreased compared to ground-reared 1g-controls ($p=0.05$; mean values 0g: 2.8ms and 1g: 4.8ms). In addition, the burst duration was slightly decreased ($p<0.1$; mean values 0g: 19ms and 1g: 23ms). These 0g-induced changes of fictive swimming resemble the motor pattern of younger tadpoles. Both, 3g- and 0g-effects disappeared within a few days after the termination of altered gravity. - CONCLUSIONS: (1) Altered gravity affects the development of the locomotor system in a reversible manner. (2) There are evidences that 3g- or 0g-induced changes of fictive swimming have a vestibular origin. (3) The changes of macular activity during altered gravity exposure may affect the development of descending projections from the brainstem to the spinal cord.

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Biomechanics of free flight control in *Drosophila* 279

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The aerodynamic mechanisms underlying flight force generation in insects are now well understood, both theoretically and from experiments with dynamically-scaled robotic models and flow visualization techniques. The intriguing question has now become how insects control the force output to stabilize flight and perform maneuvers. Quantitative analysis of flight control aerodynamics requires a precise measurement of wing and body kinematics, and a method for translating those motions into time-varying aerodynamic forces. Although it is easier to measure wing kinematics on tethered animals, this procedure deprives an insect of essential sensory feedback and prevents it from exhibiting its full behavioral repertoire. We therefore filmed saccadic turning maneuvers of free-flying fruit flies (*Drosophila melanogaster*) using infra-red 3D high speed (5000 fps) video. We then reconstructed the 3D motion of the body and both wings from six complete image sequences, each containing a saccade, and played the wing motion back through a dynamically-scaled flapping robot to obtain the instantaneous aerodynamic forces.

The measured wing motion varied surprisingly little, even during saccades. This 'generic' stroke pattern consists of a U-shaped wing trajectory, which produces a high force peak around the middle of each half stroke. A particularly pronounced vertical plunge of the wing at the start of the upstroke is responsible for the main contribution to lift and for a high lift-to-drag ratio. Unsteady aerodynamic effects are relatively unimportant, or even absent, as in the case of the clap-and-fling mechanism. Furthermore, muscle power calculated directly from the instantaneous aerodynamic and inertial wing forces was found to be at least 25% higher than previous estimates.

In accordance with the small changes in wing kinematics, the mean forces generated by a fruit fly remain relatively fixed with respect to the body. Slight differences in the force output of each wing generate enough torque, however, to change the orientation of the body and with it the direction of the force vector, just as a helicopter increases thrust by pitching downward.

To explore the dynamics of flight quantitatively, we compared the aerodynamic yaw torque produced by the wings with the resulting changes in the yaw angle of the body over the course of a saccade. We expected torque to follow the time-course of yaw velocity, in accordance with the assumption that flies must primarily overcome frictional forces. Instead, torque closely correlated with yaw acceleration, indicating that a saccading fly must primarily overcome body inertia. This surprising result is further confirmed by a calculation of torque based on the measured body kinematics and theoretically derived values for body inertia and skin friction.

To initiate a saccade, the fly tilts its stroke plane backward and increases the stroke amplitude of the outside wing with respect to the inside wing, possibly assisted by small changes in angle of attack. At the end of the saccade, this pattern is reversed to generate counter-torque and actively decelerate. The results provide new insights into the flight

dynamics in insects, with important implications for future research into the neural mechanisms underlying flight control.

280 Using local positive feedback for compliant motion in a multi-joint limb

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When limbed systems come in contact with other bodies or the substrate during their operation, a pure kinematic control is not sufficient to prevent the system from harmful collisions. The latter can occur when a single arm is used for problems like turning a crank, when multiple robot arms perform a task cooperatively and when a walking machine touches the ground with its feet. In these cases at least a combination of position and force control has to be utilised. Such a combined approach is commonly included in the superordinate concept of *compliant motion*. A classical solution for a compliant motion problem includes calculations for direct and inverse forms of both kinematics and dynamics. All this has to be done for several degrees of freedom. Especially in walking systems which are our main concern of study, the computational costs are quite high. As an alternative solution we propose to apply local positive feedback on the single joint level. This concept is robust against changes in the geometry of the mechanical system even when adding further joints. The key idea of this concept is the exploitation of the physical properties of the system's interaction with its environment. Prerequisites are the incorporation of elastic properties for the actuators as well as the integration of force and position transducers into the acting system. By introducing feedback loops which use the information provided by the sensors, this system can perform step movements which are highly adaptive to the overall movement of the robot, although the control strategy is highly decentralised. In the experiments, the six legged walking machine *Tarry 2B* was used, which is modelled on the anatomy of the stick insect *Carausius morosus*. One single leg of this robot consists of three joints, the body coxa (α-), the coxa trochanter (β-) and the femur tibia (γ-) joint. The α- and γ-joint and the foot plate of a single leg have been equipped with springs which, in combination with Hall sensors and magnets, act as force, momentum, angle and distance sensors. The spring system allows a joint to be adjusted passively by an external force even though the servo motor has not changed the angle of its axle. The result is a passive elastic bending between the two adjacent segments forming that joint. This bending together with the actual angle of the servo axle minus the old joint position results in a differential angle per time step. This angular velocity is fed back into the system's input. Because of this positive feedback the control system is at the most marginally stable. Therefore keeping the system in a usable state bears additional problems which are currently under investigation. It could be shown that a single leg is able to walk on a treadmill. Currently we apply our control strategy to the problem of turning a crank, a classical benchmark for compliant motion.

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Properties of delayed rectifier-type currents in cells of the pyloric circuit of the STG in the spiny lobster, *Panulirus interruptus* 281

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Delayed rectifier-type currents ($I_{K(V)}$) have characteristic fast time constants of activation, and deactivation and generate sustained, non-inactivating currents. They are known to play important roles in shaping spike frequency in cells that fire repetitively. In invertebrates these currents are encoded by the *shaker*, *shab* and *shaw* subfamilies of *Shaker* potassium channel genes. The pyloric network in the stomatogastric ganglion (STG) of the spiny lobster forms a neuronal network that controls the rhythmic contraction of the pylorus, the posterior part of the crustacean foregut. It is made up of 13 motor neurons of five different classes, and one interneuron, all of which show Shab and Shaw immunoreactivity in their cell bodies.

We report the properties of $I_{K(V)}$ in different pyloric cell types, studied under two-electrode voltage clamp in the isolated STG. We found that the sustained non-inactivating current has a high threshold of activation, and is made up of at least two components. These two components are present in all pyloric cell types. One of the components activates at potentials above –25mV, while the second component appears only at very depolarized voltages and does not saturate at voltage steps up to +45mV. The magnitude of the components varies among cell types but also shows considerable variation within a single type. The composite current can be fitted by a standard Boltzman equation with an added exponential function. The effects of the potassium channel blockers 4-Aminopyridine (4-AP) and tetraethylammonium (TEA) as well as quinidine on the composite current are being investigated at various concentrations. The blockers and digital subtraction are used to characterize the parts of the total current sensitive to single blockers. The low threshold component appears to be selectively sensitive to TEA (5-20mM) in some of the pyloric neurons.

Contribution of Intra- and Intersegmental Signals to the Generation of Fin Motoneuron Activity in the Lamprey Spinal Locomotor Network 282

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A functional movement pattern for locomotion relies on the coordinated activation of various sets of muscles supplying the locomotor organs. While detailed knowledge is presently available on the basic neural mechanisms underlying the generation of rhythmic locomotor activity for walking, flight and swimming, (e.g. Orlovsky et al. 1999; Oxford Univ. Press) the knowledge on the mechanisms underlying the coordination of action in different sets of motoneuron pools is fairly restricted. During swimming of a lower vertebrate, i.e. the lamprey, two groups of muscles generate propulsion and con-

trol body orientation. Those are the trunk or myotomal muscles and the dorsal fin muscles. While detailed knowledge exists on the generation of rhythmic activity in myotomal muscles and motoneurons during swimming (Grillner et al. 1998; Brain.Res.Rev.26:186), little is known about the mechanisms that organize activity of fin motoneurons (fMN) in the locomotor cycle (Buchanan & Cohen, 1982; J. Neurophysiol. 47:948; Shupliakov et al. 1992 J.Comp.Neurol.321:112). Here, we investigate the control of fMN activity by recording their activity pattern both intra- and extracellularly during fictive swimming in the isolated lamprey spinal cord induced by NMDA (0.050-0.15mM). fMNs were labeled in the spinal cord with dye (FITC) by means of retrograde transport after injection of the dye into the fin muscle 10-14 days prior to the experiment and visualized under blue light *in vivo*. Within the locomotor cycle, fMNs are always activated in clear antiphase to the ipsilateral myotomal motoneurons. Intracellular recordings revealed two different types of membrane potential modulation in the swimming cycle: while one group of fMNs showed a cyclic modulation in membrane potential that was basically similar to the myotomal motoneurons, however, with a phase shift of about 150 deg (N=15), in the second group of fMNs regularly a two to three fold faster modulation in membrane potential was superimposed on the basic anti-phase modulation (N=7). The present knowledge on topology and action of the spinal CPG for swimming in the lamprey cannot account for the generation of rhythmic activity in fMNs. Therefore, we investigated the role of intrasegmental and intersegmental signals on the generation on rhythmic membrane potential modulation in fMNs. We first tackled this question by introducing specific lesions to the spinal cord. Cutting the spinal cord along the midline did not abolish suprathreshold activation of fMNs in the locomotor cycle (N=6) and it did not affect the basic rhythmic modulation of the membrane potential in the swimming cycle (N=5). However, following sagittal cutting the peak-to-peak amplitude in modulation significantly decreased on average by about 30%. The same was true when extending the cut one segment further caudad and rostrad. Cutting the spinal cord hemi-transversal along the border to the next rostral or caudal segment did not abolish the modulation in membrane potential. Our results indicate that the rhythmic synaptic drive that patterns fMN membrane potential in the locomotor cycle does neither exclusively arise from the contralateral side of the segment nor exclusively from anterior or posterior neighboring segments. Thus, we conclude that these inputs derive primarily from intrasegmental sources.

283 Generation of Alternating Motoneuron Activity in the Deafferented Stick Insect Walking System

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We have shown recently that the rhythmic activity pattern of leg motoneurons (MNs) of a stick insect walking on a treadmill is generated by alternating depolarizing and hyperpolarizing synaptic inputs (Schmidt et al. 2001; J Neurophysiol 85: 354). In isolated deafferented thoracic ganglia, application of the muscarinic agonist pilocarpine evokes alternating rhythmic activity in extensor and flexor tibiae MNs controlling the femur-tibia (FT-) joint. The rhythmic activity pattern is formed by phasic inhibitory synaptic

inputs in conjunction with a tonic depolarization (Büschges 1998, Brain Res 283: 262). Here we explore how central pattern generating networks contribute to the patterning of MN activity when alternating activity in the antagonistic MN pools of the FT-joint is evoked in a deafferented preparation in the absence of pharmacological agents.

Alternating bursts of action potentials in extensor tibiae and flexor tibiae MN pools in the deafferented mesothoracic segment were evoked by means of tactile stimulation of the abdomen or the antennae. Activity of tibial MNs was recorded extracellulary from the leg nerves and intracellularly from their neuropilar processes in the mesothoracic ganglion. When the locomotor system was active, we investigated the dependence of membrane potential modulation in the MNs on depolarizing and hyperpolarizing synaptic inputs by injection of current into the MNs. At their normal resting potential, flexor and extensor tibiae MNs generated alternating cycles consisting of bursts that were terminated by strong inhibitory synaptic inputs. The amplitude of the inhibitory potentials was increased by injection of depolarizing current. Hyperpolarizing current injection into a MN decreased the amplitude of the inhibitory inputs and revealed a tonic depolarization of the MN during the bout of rhythmic motor activity. The modulations in membrane potential were accompanied by marked changes in the input resistance (R_{in}) of the MNs, with R_{in} being minimal during the hyperpolarizing phase of the membrane potential oscillation. We also observed a tonic depolarization of tibial MNs of the deafferented mesothoracic ganglion during sequences of walking movements in the ipsilateral frontleg, indicating that tibial MNs are tonically depolarized when the locomotor system is activated. In summary, these results suggest that during walking, central neural networks affect leg motoneurons by providing phasic inhibitory and tonic excitatory synaptic inputs.

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Intersegmental coordination of walking in the stick insect *Carausius morosus*: The influences of a single walking leg on the motoneurons of the other segments

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Behavioral studies have identified several mechanisms of interaction between neighbouring legs that contribute to the generation of a co-ordinated walking pattern in the stick insect C. morosus (Cruse, 1990 TINS 13:15). These studies show that the movement of a leg is controlled by local and intersegmental neural networks and sense organs. Here we are interested in the effects of one single leg walking on the neighbouring segments. We analysed the influences of stepping activity of one leg on the activity of leg motoneuron pools in the contralateral neighbour as well as in the neighbouring segments. We used a semi-intact preparation with either one intact front or middle leg walking on a treadmill, developed from the preparations used by Karg et al. (Karg, 1991 Biol Cybern 64:329) and Fischer et al. (Fischer, 2001 J Neurophysiol 85:341). The animal was mounted dorsal side up along the edge of a foam platform. All legs except the front or middle leg were cut in the middle of the coxa. In the segment in focus the ganglion was

de-afferented. While the animal walked with either one intact front leg or one intact middle leg on the treadmill, the activity in motoneurons innervating the protractor (ProMN) and the retractor (RetMN) coxae muscles of the thorax-coxa (TC) joint was recorded extracellularly from the respective nerves in the other segments.

(i) With one single front leg walking on the treadmill the activity of ProMNs and RetMNs of the ipsilateral mesothoracic segment was modulated in an alternating pattern coupled to the step phase of the front leg: during stance the activity in RetMNs increased, while the activity in ProMNs decreased (N=5). During leg swing this pattern was reversed. In contrast, we observed co-activation of ProMNs and RetMNs in the contralateral prothoracic hemiganglion (N=5). In all other segments walking in the front leg induced a general increase in motoneuronal activity, however, with no systematic correlation to the front leg stepping pattern (N=5 each).

(ii) During walking of a single middle leg ProMNs and RetMNs in all other segments were activated with no systematic correlation to the middle leg stepping pattern (N=5 each).

In summary, our results indicate that only during front leg walking caudal oriented influences are generated that evoke alternating activity in the antagonistic coxal MNs of a neighbouring segment.

285 Intersegmental Influences on Motoneurons and Interneurons for the Coordination of Walking Movements

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We recently described the modulation of spike activity of mesothoracic leg motoneurons (MN) of stick insects during walking activity in the ipsilateral frontleg. We used a semi-intact preparation, similar to the one described by Karg et al. (1991, Biol. Cybern. 64:329), in which a prothoracic leg was walking on a treadmill, while we extracellularly recorded from nerves of the deafferented mesothoracic ganglion on the ipsilateral side. Here we took a closer look at characteristics of this modulation by recording intracellularly from MNs in the same preparation. Furthermore, we looked for intersegmental influences on mesothoracic non-spiking interneurons (NSI) in the tibial premotor network to investigate, how coordinating signals are fed into the local segmental networks of a leg control system. NSIs were identified by their excitatory or inhibitory influence on activity of tibial extensor MNs and by their morphology, which was revealed from injection of fluorescent dye (TRITC).

We found that the mesothoracic MNs innervating the muscles of the three proximal leg joints (thoraco-coxal joint: protractor coxae (N=3); coxa-trochanteral joint: levator (N=2) and depressor trochanteris (N=6); femoro-tibial joint: flexor (N=5) and extensor tibiae (N=7); common inhibitor 1 (CI1) N=2) show a tonic depolarization of their membrane potential during walking activity of the prothoracic leg. Additionally, their membrane potential was phasically modulated with the stepping pattern of the prothoracic leg. We found evidence that this phasic modulation in membrane potential results from

additional excitatory synaptic inputs. Some of the recordings, provided evidence for phasic inhibitory synaptic inputs. For example, the fast extensor tibiae MN received inhibitory synaptic input during the stance phase of the prothoracic leg.

Intracellular recordings from NSIs (N=35) in the mesothoracic ganglion revealed a tonic de- or hyperpolarization during walking activity of the prothoracic leg. Furthermore the membrane potential of the NSIs was phasically modulated with the stepping pattern in most of the neurons. The same was true for identified NSIs that are known to be part of the local neural network controlling posture and movement of the FT-joint, the NSIs E2/3, E4 and E5/6, E8, I1. These results suggest that during locomotion intersegmental information for the control of tibial MNs does not by-pass the premotor network that processes proprioceptive signals from the FT-joint, but converges on it on the level of the premotor NSIs.

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Bilaterally Symmetrical Ventilatory Activity in Free Moving Crayfish

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The crayfish, like other decapods, is known to possess two bilaterally symmetrical ventilatory appendages (scaphognathites, SGs). Their rhythmical activity is produced by two CPGs each being located in the ipsilateral half of the suboesophageal ganglion. The question is, at what degree the activity of both ventilatory CPGs in free behaving crayfish is correlated. Temporal characteristics of simultaneously recorded right and left electroscaphognathitegrams (ESG; Goettingen Neurobiol. Rep., 1998, p.256) were studied in chronic (1-1.5 months) experiments on free moving crayfish *Procambarus cubensis* reared in the laboratory. The 10-30 second fragments of both ESGs were digitized and processed by the Statistics Graphics 6.0 programs using the Auto- and Cross-Correlation Functions. In undisturbed crayfish, long (1-2 days) staying in the experimental tank, only left or right ESG was usually observed; this correlated with long absence of locomotory activity of the animal, and its non-reactivity to external stimuli. In different crayfish, the left or the right SG could be active during several days but later it was replaced by its symmetrical analog. If the crayfish was aroused, the 'quiescent' CPG became active. The activation developed gradually in the case of external stimuli; it was rapid for mechanical stimulation of the animal (e.g. touching its carapace). In wakeful crayfish, the fluctuations of the symmetrical ESGs, spontaneous or evoked by some external stimuli, tended to be unidirectional but sometimes only one ESG was reactive. The cross-correlograms of the symmetrical ESGs in the behaviorally active crayfish demonstrated intermediate or high degree of synchronism but their parameters (value of cross-correlation coefficient and phase shift) were variable. Is is suggested that symmetrical ventilatory CPGs do not depend each other but they are under the common control of higher nervous centres, both direct or mediated neurohumorally.

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287 Neuromodulation of the Locust Frontal Ganglion Central Pattern Generator

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The frontal ganglion (FG) is part of the insect stomatogastric nervous system and is found in most insect orders. In the desert locust, *Schistocerca gregaria*, as in other insects, FG neurons innervate foregut dilator muscles and play a key role in the control of foregut motor patterns. We have previously shown that a central pattern generator (CPG) in the locust FG generates various rhythms depending on the animal's physiological and behavioural state. In addition to its role in the control of feeding-related foregut movements, the FG also plays a critical role in locust moulting. We are currently focusing on the FG CPG as a target for modulation by feeding and moult-related humoral factors, as well as by interactions with other neural centers. We employ an isolated *in vitro* FG preparation, as well as an intact *in vivo* preparation.

Application of octopamine, a well-studied insect neuromodulator, generated reversible disruption of the FG rhythmic activity *in vitro*. The threshold for octopamine's modulatory effect was 10^{-6} M, and 10^{-4} M always abolished the ongoing rhythm. Allatostatin, previously reported to be a myoinhibitor of insect gut muscles and a neuromodulator in crustacean stomatogastric ganglion, showed complex dose-dependent effects on the *in vitro* rhythm. Bath application of 10^{-8} M allatostatin resulted in a strong excitatory effect. In contrast, 10^{-7} M increased the cycle period, while application of 10^{-6} M allatostatin resulted in an almost instantaneous cease of the FG rhythmic activity. These results could also be reproduced *in vivo*, thus demonstrating a role for allatostatin as a neuromodulator in insects.

Testing for the modulatory effects of ecdysis-related peptides, we found that eclosion hormone (EH), a peptide which is instrumental in inducing ecdysis behaviour, transiently inhibited the FG rhythmic pattern *in vitro*. In contrast, application of crustacean cardioactive peptide (CCAP), previously reported to be a key peptide in the control of ecdysis-related motor patterns, induced a dose-dependent excitatory effect in an isolated FG preparation. The effects of CCAP were significantly more intense in cases where the FG was dissected out from a pre-moulting or moulting locust.

As reported for other insect CPGs, inputs from the brain were shown to have an inhibitory effect on the FG in an *in vitro* FG-brain preparation. The FG rhythmic pattern emerged only after severing the frontal connectives (FC) connecting the ganglion to the tritocerebrum. The FG rhythmic output *in vivo* is often strongly correlated with the output of the thoracic ventilation CPG. The hierarchical relation between the two CPGs was found to be mediated via the FC. Stimulating the ventral nerve cord between the thoracic ganglia generated spikes in the FC nerve. In addition, we have traced neurons situated in the suboesophgeal and thoracic ganglia up to the metathoracic ganglion after backfilling the FC.

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Acoustic pattern recognition in crickets: A template matching mechanism? 288

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Common concepts of acoustic feature extraction within the auditory pathway of vertebrates and insects assume temporal filters tuned to particular periodicities. Crickets respond selectively to the conspecific song pattern and reveal a bandpass characteristic, which is thought to arise from a set of temporal filters tuned to a restricted range of periods (Schildberger, K (1984) J. Comp. Physiol. A: 155,171 - 185). As shown by behavioural experiments, two closely related species (Teleogryllus) differ in fundamental filter properties which raises the question of how so distinct properties of homologous neuronal circuits for pattern analysis have evolved during speciation. Signal analysis by cross-correlation offers a simple explanation for differences in pattern selectivity between the two species. Cross-correlation determines the similarity of an external pattern with an internal template in a certain time window. A comparison of behavioural data and cross-correlation values suggests that both species have very similar templates. Solely a change in the evaluation time window is required to account for the observed differences in feature extraction that serves to maintain species isolation.

The "What" and "How" of temporal integration in an insect auditory system 289

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Sensory neurons integrate over a certain stimulus duration for producing their responses. This temporal integration is particularly critical for auditory neurons as acoustic stimuli inherently contain most structure and information in the temporal domain.

For example, whereas sensitivity to fast transients in the signal suggests short time constants, frequency selectivity requires extensive averaging by long time constants. Therefore, the interplay between the mechanical characteristics of the sound receiver (e.g., cochlea, basilar or tympanal membrane) and neuronal properties (e.g., gating of transduction channels, membrane time constant) is of particular interest in order to understand the encoding of these different stimulus features.

We recorded responses of single auditory receptor neurons in the auditory nerve of *Locusta migratoria* to short (sine-tone) and ultra-short (click) stimulation of different temporal composition. By means of online analysis of the electrophysiological data and an internal feedback loop to the stimulus generation, we identified such stimulus classes that lead to the same response probability (spike probability).

Asking first, "what" stimulus attribute is integrated, we find that this is best described by the square of the stimulus amplitude. This allows us to extend a previously found de-

scription of spectral integration based on "energy detection" (Gollisch et al., J. Neurosci. 2002) to the time domain.

Subsequently searching for "how" temporal integration can be described and which time scales are relevant, we varied the gap between two click stimuli. This shows that integration is extremely fast (within less than 1 msec), which might be connected to the fact that fast fluctuations in the natural stimulus environment are much more important for the animal's behavior than sound frequency discrimination (von Helversen, J. Comp. Physiol. 1972). In addition, the spike probability shows resonance on roughly the same time scale. This most likely follows from the resonance properties of the tympanal membrane (cf., Michelsen, Z. vergl. Physiol. 1971).

The resonance frequencies and the Q values obtained from the spike probabilities correspond well with the independently measured frequency tuning of the receptor cells. The results are combined in a model description. The coupling of the resonance to the appearance of spikes illustrates how temporal integration in these cells is directly connected to their frequency selectivity.

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290 Coding capacities of auditory receptor cells under different stimulus conditions

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Auditory signals are characterized by a complex temporal structure. Does the amount of complexity that is preserved in a neuronal answer depend on the specifics of the signal? To answer this question, we compute the mutual information between different acoustic signals and the responses of auditory receptors of the locust, *Locusta migratoria*. The mutual information serves as a measure of the reliability with which the features of a specific signal are represented in the spike trains. We are investigating to what extent auditory receptor cells employ the complexity inherent to the auditory signal in order to encode the stimuli received by the system.

Grasshoppers of the group Acrididae use a highly specialized system of producing and receiving auditory signals in order to attract and find a mate. These "mating songs" have a specialized temporal structure which seems to be of greater behavioral significance than the frequency content of the signal. Due to this temporal structure the signal contains a stereotypical, bi-modal amplitude distribution.

In order to investigate the significance of the natural signals' dynamics on the encoding by the nervous system, we have focused on receptor cells, which transduce the auditory signal from the auditory organ into the nervous system. Prior investigations using a stimulus reconstruction method (Machens et al., J. of Neurosci., 2001, pp. 3215-3227) have shown that higher information rates and coding efficiencies can be obtained for signals with the natural-songs' amplitude distribution and time-scale. In this work we focus on the properties of the stimuli that lead to this precision.

We apply the direct method for the estimation of information rates and coding efficiencies (Strong et al., Phys. Rev. Let., 1998, pp. 197-200) to this system. This has the advantage of making no assumptions about the coding scheme of the system in question. By comparison with the reconstruction method we gain insight into the importance of non-linear coding mechanisms under natural conditions.

We record the responses of receptor cells to different types of stimuli. From the time series of action potentials we estimate the mutual information. We vary the statistical properties of the amplitude distribution of the stimuli along two different dimensions: its variance and the form of the distribution: a bi-modal, natural song like amplitude distribution versus a gaussian amplitude distribution.

We find that an increase in variance leads to a significant increase of coding efficiency and information rate. Furthermore the effect of the signals' amplitude distribution is quantified. The comparison of the direct method and the stimulus reconstruction technique allows us to identify small but considerable contributions of non-linear coding.

The results suggest a simple quasi-linear encoding by the receptors that might be explained by a fast temporal integrator (see poster by Gollisch and Herz)

Discrimination of Natural Grasshopper Songs by Auditory Interneurons

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A basic problem of all sensory systems is the correct discrimination and classification of natural signals. These tasks are impeded by noise working on two levels: (1) Environmental noise degrades any signal to a certain extent. (2) Additionally, the processing of signals within the sensory system is subject to various stochastic processes. Here we investigate the effect of this second noise source on the ability of grasshoppers to discriminate acoustic communication signals. For many grasshopper species, mate finding relies on the recognition of the species-specific stridulation signals. Female grasshoppers need not only classify into "correct" (conspecific) and "incorrect" (heterospecific) signals, but also disriminate among the different conspecific signals due to sexual selection. Indeed, recent studies have found an astonishing capability of auditory receptors in female grasshoppers to discriminate between conspecific songs (Machens et al. 2001a and submitted). In our present study, the responses of second and third order interneurons were analyzed, with emphasis on the following questions: (i) How does the discrimination capability of interneurons compare to that of receptor cells? (ii) How does discrimination relate to the extraction of certain auditory features?

In order to test the discrimination performance, a random sample of eight calling songs recorded from different individual male grasshoppers (Chorthippus biguttulus) was used. The songs differ in several clues, (i) the syllable duration, (ii) the pattern of amplitude modulation within the syllables, and (iii) the frequency content. A successful discrimination might therefore be based on each of these cues. In order to eliminate two of the differences, we used a second set of songs in which the syllable duration was

rescaled to a common length and the carrier spectrum was equalised. Hence, if at all, these rescaled songs can be discriminated only on the basis of the amplitude modulation pattern within the syllables. The responses of interneurons were recorded intracellularly while repeatedly presenting the songs. The (dis)similarity between the elicited spike trains was calculated by using a distance measure introduced by van Rossum (2001). Once the distances between all the spike trains have been calculated, a clustering algorithm enables to quantify the discrimination success.

Among tonically responding local interneurons, we found a similarly high discrimination performance as in receptors. In contrast, the discrimination success dropped when based on the spike trains of ascending neurons. Since these neurons tend to extract certain acoustic features and thereby omit part of the information present in a receptor's spike trains, perfect discrimination might be impossible on the level of a single ascending interneuron. It remains to be elucidated how the discrimination success can be enhanced by combining the information content of several ascending neurons. Interestingly, the time scales at which the performance works best for receptors and interneurons was consistently between 3 to 15ms. Hence, this may represent a time constant which is basal for the processing of behaviourally relevant auditory information in these insect.

292 Variability in spike trains of locust auditory receptor neurons under constant and dynamic stimulation

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Neurons exhibit variability in their responses to repeated presentations of the same stimulus. This response variability is tightly connected to the nature of the neural code. If spike trains are highly deterministic, information can be carried by every spike, whereas if they are highly stochastic, the read-out must contain some sort of averaging, either over time or over a neuronal population.

The auditory system of acridic grasshoppers is well suited to assess these aspects of neural coding with respect to a specific task: Despite its relatively simple architecture with only a few hundred neurons, the auditory system plays an important role in the recognition of species-specific calling songs and mate finding. The neurons must therefore encode the relevant acoustic cues faithfully.

We examined the variability of spike trains of single locust auditory receptor neurons in response to different acoustic stimuli. Spike trains were recorded intracellularly from the neurons' axons in the auditory nerve. We used two sets of stimuli, constant and amplitude modulated pure tones.

As shown by our data, the variability under constant stimulation strongly depends on the firing rate evoked by the stimulus. Different intensities of the same tone were therefore played to cover the whole range of firing.

Interspike interval (ISI) variability as quantified by the coefficient of variation (CV) is highest for low firing rates (below 50 Hz) with values near unity, and it decreases with

increasing firing rates down to CV values of about 0.2 for maximum firing (around 300 Hz).

We propose absolute and relative refractoriness as a possible explanation of this finding: The different shapes of the ISI distributions at different mean firing rates can be modeled using a single phenomenological "recovery function" which estimates the time course of the neuron's relative excitability directly after a spike.

In a second set of experiments, we stimulated the receptor cells with amplitude modulated pure tones and focused on the variability of the spike count using the Fano factor as a measure.

For these dynamic stimuli, we observed a similar dependence of the variability on the firing rate as for the constant ones: periods with low firing rates come with a high Fano factor, whereas episodes of fast firing lead to less variability. Furthermore, a renewal process equipped with a recovery function obtained from constant stimulation leads to a good prediction of the observed Fano factors. The derived model for spike-train variability is thus applicable to constant as well as dynamic stimulation.

This study gives a quantitative example of how variability is affected by the neural firing rate and refractoriness. Previous studies on stimulus encoding by locust auditory receptor neurons have examined adaptation and spectral integration (Gollisch et al, J. Neurosci. 2002), but they have focused on average responses. A combination of those results with the present model will lead to a more realistic description of stimulus encoding.

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The effects of stimulus rise time on temporal modulation transfer functions 293

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A characteristic feature of many acoustic signals are rapid amplitude fluctuations. Hence, a fundamental prerequisite for signal recognition is an adequate temporal resolution of the neural network. Temporal modulation transfer functions (tMTF) are a widly used tool to investigate the limits of temporal resolution of auditory systems. In this study we focused on the question of how the tMTF depend on the shape (i.e. the rise time) of sound pulses. Furthermore, we investigated how different duty cycles affect the temporal resolution.

While recording intracellularly from identified auditory interneurons of Locusta migratoria we presented acoustic stimuli whose amplitudes where modulated either sinusoidally or rectangularly with a duty cycle of 0,5, in a frequency range of 10 to 500 Hz and modulation depths of 100, 50, 25 and 12,5%. In another set of stimuli the sine shaped amplitude modulation was stepwise transferred into one with rectangular onset. Finally, the duty cycle of the rectangular pulse pattern was varied between 0,2 and 0,8.

Most of the neurons exhibited low pass filter characteristics in their tMTFs under both stimulus conditions. However, tMTFs often showed a lower modulation threshold for rectangularly modulated stimuli at lower frequencies. From tMTFs minimal integration times (MIT) can be calculated which are an indicator for the temporal resolution. The MITs found for auditory interneurons had values from 0,6 ms to ~ 6 ms, and thus covered a larger range than found in auditory receptors (Prinz and Ronacher 2002, J Comp Physiol A 188: 577-587). Interestingly, in two types of cells the neuronal response depended strongly on the shape of the sound pulses. In an ascending interneuron we found a pronounced shift of the minimal integration time from 2,7 ms for rectangularly modulated stimuli compared to 6,4 ms for the sine wave stimuli. Another type of neuron responded highly selectively to sinusoidal stimuli in a frequency range of 10 to 125 Hz. Sound pulses with sudden onsets elicited almost no spiking response in this cell while the spike count increased with longer rise times. At higher frequencies all stimulus onsets become steeper, and, consequently, the specific effect of rise time disappeared in the neuronal response. Hence, this neuron acts as a rate filter for graded onsets. Remarkably, the shape of the tMTFs and the minimal integration times obtained from either sinusoidally or rectangularly modulated stimuli were almost identical for this cell.

In general, auditory interneurons exhibited a much better phase coupling at smaller duty cycles. Because the sound energy in these stimuli is low and concentrated in a certain time window less spikes occurred which resulted in an improved phase coupling compared to higher duty cycles.

Our findings demonstrate that the application of standard tMTFs is not always optimally suited to reveal the real processing capacities of auditory neurons.

294 Processing by prothoracic auditory interneurons - a basis for changes in calling song responsiveness of female crickets: A comparison of three species.

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L3, an auditory interneuron in the prothoracic ganglion of the female cricket, *Acheta domesticus* responds to models of the male's calling song (CS) in two different ways: a phasically encoded immediate response; a more tonically encoded prolonged response. The onset of the prolonged response required 3 - 8 sec of stimulation to reach its maximum spiking rate and 6-20 sec to decay once the calling song ceased. It did not encode the syllables of the chirp. The prolonged response was sharply selective for the 4-5 kHz carrier frequency of the male's calling songs and its threshold tuning matched the threshold tuning of phonotaxis, while the immediate response of the same neuron was broadly tuned to a wide range of carrier frequencies. The thresholds for the prolonged response covaried with the changing phonotactic thresholds of 2- and 5-day-old females. Treatment of females with juvenile hormone reduced the thresholds for both phonotaxis and the prolonged response by equivalent amounts. Of the 3 types of responses to CSs provided by the ascending L1 and L3 auditory interneurons, the threshold for L3's prolonged response, on average, best matched the same females phonotactic threshold. The prolonged response was stimulated by inputs from both ears while L3's immediate

response was driven only from its axon-ipsilateral ear. The prolonged response was not selective for either the CS's syllable period or chirp rate.

The prolonged response has not yet been described for the responses of auditory interneurons in other gryllid species. The AN2 neuron of female *Gryllus bimaculatus* does produce a prolonged response as well as an immediate response to model CSs and thus is homologous to the L3 neuron of *A. domesticus*. However, the prolonged response of the AN2 neuron of *G. bimaculatus* is quite variable from animal to animal and can change substantially during a recording from a single female. The responses of AN2 neurons with well developed prolonged responses are quite comparable to those of the L3 neuron. The relatively large variability in the prolonged responses of the AN2 neuron suggests that regulatory influences are different between animals and also vary within a single animal. Application of picrotoxin frequently causes an AN2 neuron with little prolonged response to develop a strong response within a few minutes. By comparison, AN2 neurons of female *G. pennsylvanicus*, exhibit a prolonged response which is less variable than for *G. bimaculatus*, but is more variable than for *A. domesticus*. The role of inhibition in shaping this response in female G. pennsylvanicus is also being evaluated.

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Short term changes in calling song recognition and its underlying neuronal processing: A comparison of three cricket species

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Female crickets respond behaviorally to the calling song (CS) of distant males by walking toward the source of the call. This phonotactic behavior is tuned to the syllable period (SP) of the male's CS. The L3 neuron in *Acheta domesticus* selectively processes SPs, providing young females with information that is used to identify the conspecific male's call - leading to phonotaxis. In old females this selective processing is frequently lost, resulting in unselective phonotaxis to calls that could represent the males of several other species. Changing the behavioral conditions (e.g. changes in visual or olfactory input) can evoke immediate changes in the old female's phonotactic selectiveness for the SPs of model CSs. These changed conditions also can induce changes in the selective processing by L3 that underlie selective phonotaxis. The AN2 of *Gryllus sp.* is the structural and functional homolog of the L3 neuron. In the European species, *G. bimaculatus*, and the American species, *G. pennsylvanicus*, the AN2 also selectively processes the SP of model calls. However, its responses can be quite variable from animal to animal in both species, especially to CSs with SPs that are shorter than the natural SP. The correlations between the selective processing of the AN2 neuron and the selective phonotaxis of females of both species will be demonstrated and evaluated. The role of inhibition in the selective processing by the AN2 of each species is evaluated by showing the influence of picrotoxin, a blocker of inhibitory input. The results demonstrate that changes in the inhibition that influences the AN2 neuron result in selective

processing that correlates consistently with the selective phonotaxis that is typical of females of both species.

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296 Short Term Changes in Calling Song Recognition of Crickets and its Underlying Neuronal Processing: Pharmacological Evaluation.

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Young adult female crickets (*Acheta domesticus*) are attracted to calling songs which have syllable periods that match the conspecific song (50 -70 ms). As the cricket ages, the range of attractive syllables increases (30-100ms - ie the female is less selective to syllable period). The L3 neurons in the prothoracic ganglion are also selective for syllable period in young females and are not selective in old females. The syllable period selective response of L3 is caused by an ipsilateral delayed inhibition which results in maximum decrement (response of the third syllable is about half that in response to the first syllable of the chirp) and correlates with the selective behavior of these same females. L3s in old females are not selective (a reduced responsiveness to all syllable periods that does not decrement much). The age- related change in selectivity is the result of increased contralateral inhibition by the ON1 neuron in old females which is less coupled to L3 in young females.

Application of picrotoxin to old unselective females can bring on more selective responses within 2-6 hours following injection. Picrotoxin applied to the prothoracic ganglion while recording from the L3 of old females, results in more decrement in the response and thus more selectivity in L3 within minutes (changes seem to be greatest at the shorter syllable periods). Picrotoxin does not seem to effect the presence of the delayed inhibition onto L3 (which causes syllable period selectivity) but does change the unselective response of L3 in old females.

Since evidence suggests that ON1 is histaminergic and since ON1 is implicated in the selectivity of L3 and behavior, we have begun to evaluate whether the syllable period selectivity of young and old females are sensitive to histamine or histamine antagonists. Preliminary evidence suggests L3 responses can be modified within minutes by histamine and histamine blockers. Behavior correlates to these changes are also being evaluated.

The effect of single cell killing in the auditory network of a bushcricket, *Ancistrura nigrovittata* (Orthoptera: Phaneropteridae)

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Analysis of neuronal function benefits from techniques that allow for the selective removal of identified neurons or target cells. Methods for ablating neurons fit into two broad classes: 1) toxigenic ablations and 2) physical ablations (Mountcastle Shah & Jay. 1993. Curr Opin Neurobiol. 3:738-742).

For studying the auditory network in the prothoracic ganglion of Ancistrura nigrovittata we are using a physical ablation method (dye-mediated photo-ablation) in which radiant energy of 442nm from a He-Cd Laser is selectively targeted to a cell stained with Lucifer yellow CH (LY). The excitation of the dye results in photochemically induced cell death without affecting overlying or neighboring cells (Miller & Selverston. 1979. Science. 206:702-704).

Control experiments with intracellular recordings in cells filled with LY (5% in 1M LiCl) by means of iontophoresis by a negative current of 0,3 to 0,4 nA for 3 to 5 minutes and exposed to the laser light have demonstrated an increase in cell activity and irregular spiking behavior during the first 20 seconds of exposition and complete disappearance of all cellular activity including the membrane potential after 30 seconds. Such results have been observed in receptors, ON1, AN1, TN1 and TN3.

Control experiments with intracellular recordings either in cells filled with Neurobiotin (5% in 1M KAc) by means of iontophoresis with a positive current of 0,2 nA for 3 to 5 minutes or with cells recorded with electrodes filled with 3M Potassium Acetate have showed normal activity during at least the first 3 minutes of laser exposition. After that time or later ON1, AN1, AN4 and TN1 interneurons started to decrease activity until one moment in which the activity completely disappeared.

Experiments were conducted with filling the first cell with LY and killing it by 1.5 minutes laser exposition while recording intracellularly the second cell with Neurobiotin. Epifluorescence microscopy of ganglia fixed with paraformaldehyde, dehydrated and cleared with methylsalicylate, after revealing the Neurobiotin staining with streptavidin-Cy3, have confirmed the presence of the first cell stained with LY and the second cell stained with Neurobiotin. A major if not complete reduction of contralateral inhibition was observed in experiments with killing one ON1 while recording from its mirror image partner. This was demonstrated by a loss of IPSPs in dendritic recordings and by an increase of spike number with soma-contralateral stimulation. A reduction of contralateral inhibition was also observed in experiments with killing ON1 while recording from the soma-ipsilateral AN1 and TN1. Little effect has been seen so far when killing a single receptor while recording from an interneuron. No effect has been seen when

killing a DUM cell while recording from AN1, even though DUM cells have been hypothesized to be a prime candidate for frequency specific inhibition of AN1 (Stumpner. 2002. J Comp Physiol A. 188: 239).

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298 A method for correlating neuronal responses to sound signals in complex habitat noise

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The nocturnal neotropical rainforest constitutes a challenging problem for all hearing animals due to the intense masking noise level. Calls of many individuals and species of crickets and frogs add to an intense frequency band below 8 kHz (LF-noise), whereas calling songs of bushcrickets and echolocation signals of bats extent the noise into the higher sonic and ultrasonic range up to 100 kHz. We studied the neuronal representation of signals in the spike activity of identified neurons of the afferent auditory pathway under such masking conditions using outdoor neurophysiological methods [1].

However, the identification of those signals eliciting a neuronal response from those that do not within such a complex acoustic environment represents a challenging analytical problem. Reverse correlation methods [2] assuming an independent random stimulus ensemble cannot be applied successfully, due to permanent loud LF-noise and a high degree of signal interference at higher frequencies. We therefore developed a method for the identification of signals represented in afferent spike discharges within habitat noise, by cross-correlating the spike density function (SDF) with the log of the amplitude modulation of a filter bank output derived from a spectrographic decomposition of rainforest noise. Further, the onset and the end of an increase in the SDF provides cues about the time domain of represented signals. This method is based on the tonic discharge properties of the neuron under study (ω cell) and assumes similar amplitude modulations in both, the stimulus and the SDF of the response. Similarity between subsequent segments of stimulus and response was calculated with the help of the "goodness of fit" correlation coefficient. Because the amplitude modulation of decomposed signals strongly varied in their spectral content we empirically adjusted the width of the gaussian filter kernel used for the calculation of the SDF. In order to evaluate this method, the representation of various high-frequent sound signals masked by low-pass filtered rainforest noise was analysed in playback experiments.

Applying the cross-correlation method resulted in the positive identification of most high-frequent signals. Benefits and limitations of the currently used stimulus identification method will be discussed.

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The contribution of different auditory receptor cell groups to acoustic startle responses in the locust flight. 299

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Many species of flying insects initiate defensive flight behaviours in response to ultrasound, pointing to the potent force that predation from echolocating bats has been for the evolution of hearing organs (Hoy 1992). Even non-flying insects perform acoustic startle responses like cessation of singing, jumping or freezing in response to ultrasound (Faure & Hoy 2000). In locusts, high frequency sound pulses elicit negative phonotaxis resulting in a steered flight response away from the sound source (Robert 1989). Because the behavioural tuning of negative phonotaxis correlates quite well with the tuning of the group of d-cell receptors in the locusts hearing organ it has been suggested by Robert that these receptors elicit the steering response in flight.

We tested this prediction by using a previously described method of selectively destroying the d-cell receptors in the hearing organ of locusts from outside without having access to the organ itself (Plewka 1987). This allowed direct comparison of the acoustic startle responses before and after elimination of the d-cells. In control animals, stimulation at low intensity (45-60 dB SPL) and frequencies between 20 to 40 kHz predominantly elicited negative steering with the abdomen and legs, whereas higher SPL up to 90 dB elicited various flight manoeuvres like interrupted folding of wings, cessation of flight or upward bending of abdomen. Such behaviours would result in loosing altitude or dive to the ground in freely flying insects. In operated insects without d-cells in no case negative steering was observed at any intensity. However, startle behaviour could still be elicited at higher intensities, although the probability was significantly reduced compared to the control.

Neurophysiological experiments confirmed, that the method was selective in destroying only the d-cells in the hearing organ. The threshold of the tympanal axons was unaltered at frequencies up to 10 kHz after destruction, whereas thresholds at 30 to 40 kHz shifted by about 25-30 dB to values reflecting those of the remaining low-frequency receptor groups. This correlates with the increase in the behavioural threshold of acoustic startle at high frequencies in the operated insects by the same degree. These results are taken as evidence for a neuronal network for the acoustic startle, where d-cells selectively elicit the directional, negative steering response, and the low-frequency groups (probably in cooperation with the d-cells) elicit high intensity, nondirectional manoeuvres that result in flight cessation or diving to the ground.

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300 Development of the auditory system of *Mecopoda elongata* (Orthoptera)

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The auditory sensory cells of bushcrickets are linearly arranged within the tympanal organ. This order correlates with the tuning of the receptor cells. Furthermore, the order correlates with the central projection of the sensory cells within the CNS. Thus, the precise order of the auditory system of bushcrickets makes it a good candidate for studying developmental mechanisms. Additionally, during postembryonic development the auditory system functionally differentiates from a vibration receiver to airborne sound receiver, thereby reflecting the evolution of the tympanal organ. Here we present a first study on the development of the auditory system in *Mecopoda elongata*.

At first we analysed the auditory system of the adult. The tympanal organ is typical for tettigoniids and contains about 44 sensory cells in the crista acustica. Together with the subgenual organ and the intermediate organ, the crista acustica is part of the complex tibialis organ. The sensory cells project into the prothoracic ganglion, again similar to other tettigoniids. The functions of the acoustic system are probably predator avoidance and intraspecific communication. Males produce calling songs with chirps of about 250ms duration containing a broad band of frequencies (3kHz to at least 40kHz) with a main peak around 8kHz.

For studying the development we daily collected the eggs which were deposited on the ground. The embryonic development takes about 44 days at a constant temperature of 24°C. Seven larval stages mark the postembryonic development. Larval development shows a considerable variation in duration, but also in the size of individuals. In general the first three stages take about 14 days each, thereafter each stage takes 7-10 days. The external morphology of the tympanal organ develops gradually during the postembryogenesis. Scanning electron microscopy shows the development of the tympanal membranes during the last larval stages. The sensitivity to airborne sound increases throughout postembryonic development.

During embryonic development the sensory cells of all three parts of the complex tibialis organ are generated distally of the femur-tibia joint. The linear arrangement of the sensory cells of the crista acustica develops at around 50% of embryonic development, but at this stage the crista acustica extends almost throughout the whole tibia. In later stages it is restricted to the proximal tibia. Cell lineage analysis with incorporation of BrdU shows the differentiation of scolopidial sensory cells from sensory mother cells. The cell surface molecules of the sensory cells can be immunhistochemically labelled with anti-HRP (Sigma Chemicals), anti-Lachesin (Karlstrom et al., Development 1993), but not with grasshopper anti-Fasciclin I (Bastiani et al., Cell 1987).

Axogenesis starts shortly after the generation of the first sensory cells at around 50%. The axons enter the prothoracic ganglion and arborize in the central neuropile. Individual sensory cells are analysed for the formation of the central projection. Within the developing ganglion a number of different molecules are expressed, which might be

involved in regulation of the central projection development. For example the transcription factor *engrailed* is expressed at the posterior margin of the ganglion.

Fungal control of sexual behaviour

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The auditory behaviour of insects is under control of genetic and epigenetic factors. Here I report a first case where a fungus changes an auditory behaviour.

A number of species of cicadas host fungi of the genus *Massospora*. This pathogenic fungus infects the nymphs of the cicadas as they emerge from the ground. The cicada *Okanagana rimosa* can be infected with *M. levispora* (Soper at al., Ann. Entomol. Soc. 1976), which is only one of a number of parasites and predators attacking the cicada. The cicadas start emerging in early June and some days after emergence male *O. rimosa* produce a calling song to attract female cicadas (Moore, 1966 Mich Acad Sci Lett). Females perform a flight phonotaxis to locate the males (Lakes-Harlan & Moore, in prep.). This behaviour is adapted to the temporal and spectral content of the male calling song. Males also possess an auditory sense, however, the only known behavioural functions are synchronisation of song production and involvement in alarm and escape behaviour.

Phonotaxis experiments and observations in several years in Northern Michigan showed that by early July the first fungus are visible at the cicadas. The fungus seems to effect both, females and males to a similar percentage. It develops within the abdomen and the abdomen swells. Finally, abdominal segments break and the fungus can replace all caudal parts of the abdomen, exposing the spores or conidia for distribution. Obviously, males are unable to copulate and females can not proceed within oviposition. However, females continue to perform phonotaxis. Moreover, by contrast to intact males, fungus infected males also perform phonotaxis! They have not been observed to produce calling song, although in most cases the timbal is intact and they are able to produce disturbance noises. The frequency spectrum of these noises peaks at 9.2 kHz, similar to intact males (9.0kHz). Thus, the fungus does not influence physical parameters of sound emission.

Especially males can also be infected by bacteria, nematoda and by a parasitoid fly (in the latter case with rates up to 90% of a population). However, male phonotaxis has never been observed in intact males, nor in those with parasites other than the fungus. The observed changes seemed to be specific for the fungus, perhaps facilitating the distribution of the fungus.

Behavioural changes by parasites, notably by fungi have been reported in a number of cases. However, to my knowledge, this is the first case of a change in a sexual auditory behaviour. The results show that the neuronal networks for song recognition and phonotaxis are present in both, males and females. It will now be interesting to investigate the mechanism by which the fungus is able to control a neuronal network. One hypothesis to follow is that a neuroactive substance is released by the fungus, which changes neuronal connections and activates new neuronal networks.

302 Habituation of the startle response of *Gryllus bimaculatus* (Orthoptera)

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Flying crickets display a robust avoidance behaviour: unilateral stimulation with ultrasonic sound pulses results in a posture directing the flying animal away from the sound source. This behaviour characteristically habituates: successive stimuli reduce the motoric reaction (e.g. Wyttenbach et al., Science 1996). In our experiments we analysed this plasticity in auditory behaviour especially in respect to temporal parameters.

Flying female crickets were mounted in front of a wind tunnel and laterally stimulated with sound pulses of 10ms duration and 16kHz carrier frequency. The response was registered with an opto-electronic camera and electronically analysed.

1. The response was tested with increasing sound stimuli (5dB increments). This test series revealed at least two results: (i) the threshold of the startle response was at about 60 dB SPL. This is about 10dB lower than reported in previous studies. (ii) The second finding is that despite the increasing sound pressure level the animals showed a habituating reaction. However, the habituation is discontinuous: for example at a repetition rate of 2 pulses per second (pps) the habituation curve is interrupted at 80 dB SPL - the reaction to this stimulus is stronger than that to the previous signal. This behaviour might be interpreted in the biological context of an approaching predator which is closing in on the cricket and that a stronger reaction is needed for avoidance. This discontinuity also depends on the repetition rate: at faster rates the interruption occurs at lower sound pressure levels.

2. The habituation response depends on the repetitions rate with stimuli of constant intensity (80 dB SPL). Increasing pulse repetition rates results in a faster habituation. The habituation curve consists out of two components: a rapid decrease and a basic level. At repetition rates higher than 1pps, the habituation immediately reaches the basic level.

3. If two series of pulses were delivered with a pause of 5 seconds the second series shows much faster habituation to the basic level. The first reaction of the second series is not significantly reduced compared to the initial response. Thus, one might speculate that some "memory" for the habituation occurs in the central nervous system.

4. The habituation of the second phase also depends on the duration of the pause. The pause is necessary to reset the habituation as described above, and pauses need to be longer than 2 seconds for an reset of the habituation.

The habituation reaction has further be used as a behavioural test for lesion induced plasticity in the nervous system ("metaplasticity"). One ear of adult females was amputated and the habituation evaluated in respect to the time after operation. These animals generally showed a more variable reaction than intact animals. Furthermore, the reaction does not habituate in experiments with increasing sound pressure level (see point 1. above for intact animals). Repetitive stimuli with a fixed sound intensity elicit compa-

rable habituation curves than in intact animals (points 2-4). The behaviour does not change with time after the operation.

Is the auditory sense of male *Emblemasoma auditrix* (Diptera) useless? 303

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The dipteran ear in the tachinid flies shows a sexual dimorphism (Lakes-Harlan & Heller, 1992; Robert et al. 1992). The reduced hearing sensitivity and different tuning of the male tympanal organ was hypothesised to correlate with the necessity for females to locate the sound producing host. The function of hearing in males was discussed in respect to predator avoidance of these nocturnal flies.

A second group of flies possessing a tympanal organ are day active Emblemasomatini (Sarcophagidae). The females of *Emblemasoma auditrix* locate a sound producing male *Okanagana rimosa* (Cicada, Homoptera) and deposit a larva into the host (Soper et al. 1976; Lakes-Harlan et al. 2000). Thus, females use the auditory system for the same function as tachinids. Here we report data on the auditory system of males.

By contrast to the tachinids, the tympanal organ of *E. auditrix* shows no sexual dimorphism. Scanning electronmicroscopy demonstrates that the morphology of the ear is very similar in both sexes. Furthermore, the auditory system of both sexes is tuned to the calling song frequency of the host, as revealed by extracellular recordings.

Thus, what could be the function of the male hearing system? One hypothesis is that males and females meet at the host, perhaps for mating. Male phonotaxis was occasionally observed in the field. In such cases the males often did not reach a sound emitting loudspeaker, but at least came close to it. To test this phonotaxis further, males were caught in the field and were tested in the laboratory.

In phonotaxis experiments in an arena, most males show a response to the calling song. A differentiated scoring system revealed that most males reacted to the sound (with turning towards the sound source). Some males moved towards the loudspeaker, but only a minority performed positive phonotaxis and reached the loudspeaker in 50cm distance. Thus, the experiments and observations clearly suggest that males are able to perform a phonotactic approach. However, other observations are not in favour of the hypothesis: firstly, male flies were observed early in season with almost no overlap to the occurrence of singing males of the host species. Therefore, they will almost never have the chance to hear the phonotactically relevant signal. Secondly, virgin females probably do not perform phonotaxis. Almost all attracted females carried larvae in their abdomen. Also a mating close to the experimental loudspeaker has never been observed. It was observed that males sit at sunny places and chased females flying by, as in many other species of sarcophagids.

Unfortunately, other functions of the male ear are also questionable: we found no indications for an advantage of an auditory detection system for predator avoidance in comparison to other sympatric sarcophagid species. We also could not identify any other

sound source in the biotope, which might be of biological relevance. Thus, our current hypothesis is that due to some genetic coupling the evolution of the hearing sense took place in both sexes, although it is essential only for female behaviour.

304 Phonotaxis of *E. Auditrix* using discontinuous signals

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Many insects show phonotactic behaviour in response to sexual signals, like calling songs. Production of such a signal is influenced by sexual selection as well as by selection pressure from predators. For pure intraspecific communication the emission of signals should be as long as possible. On the other hand, enduring signals could pose a thread to the sender due to enhanced chances for a predator to locate the sender. The duration of an acoustic signal should reflect a balance of the different aspects. But both species, the conspecific and the predator should be able to deal with the given duration of the signal. In the case of acoustically hunting parasites this means that their search strategy should be adapted to the signal production of the host. Two extreme reactions could be hypothesised if the signal duration is not sufficient to home in on the host: (i) the parasites could stop searching at the end of the signal, wait and continue host location after the next onset of the calling song. (ii) The parasitoid could immediately leave the track and start searching for other calling hosts.

We investigated the search behaviour of the parasitoid fly *Emblemasoma auditrix* in the field and the laboratory. The parasitoid uses the calling song of the cicada *Okanagana rimosa* to locate the host (Soper et al., Can J Zool 1976; Lakes-Harlan et al., Zoology 2000).

We found that in the field individual cicadas produce songs from one second to some minutes, depending on weather conditions, population density and season. Especially in low density populations and with sub-optimal weather conditions acoustic signals are produced only at a low rate and with a short duration. At least in such situations, it is easily conceivable that the parasitoid starts performing phonotaxis, however, that the signal ends before the host is located. Therefore we mimicked this conditions experimentally (signal duration 500ms - 10s with pauses of 1s - 20s)

If a continuous signal is stopped at the arrival, the animals waited for periods up to six minutes at the place where they last heard the signal. Many of them started to perform short walks or flights in the close surrounding. This behaviour might be interpreted as search behaviour, because during these movement phases they often returned to the starting place.

If short discontinuous signals were tested in the field, many parasitoids were still able to locate the loudspeaker. Signals of one second duration (with pauses of 10 seconds) were sufficient to attract flies. During the pauses most flies rested, but they also could perform search movements, again often with a return to the starting point.

In laboratory experiments the finding from the field were confirmed in a controlled surrounding. The flies often rested during pauses of the signal and performed phonotaxis

during the signal. If the duration of directed movement towards the speaker was compared between tests with continuous and discontinuous signals both were rather similar.

In summary, the parasitoid seems to be adapted to the signal conditions found in the field.

Processing of sounds by sensory cells and interneurons: The insect as a model for vertebrates? 305

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Processing of sounds for recognition or localization is an important task for most vertebrates and many invertebrates. Here I present data from bush crickets demonstrating some similarities in the construction and function of the auditory pathway of insects and vertebrates. This may help to attract the interest of researchers working on vertebrates, since insects are more easily accessible for studying principles of sound processing.

The structure of the bushcricket's sensory organ reminds of the mammalian cochlea. It comprises a series of sensory cells, which are tuned to different frequencies (the more distal, the higher the best frequency). Their terminations exhibit a tonotopic projection into the segmental ganglion of the CNS. The huge advantage of this sensory organ as compared with the mammal cochlea is the small number of sensory cells (20 to 60), which can be individually identified by their position in the organ <BP Oldfield (1988) Trends Neurosci 11: 267, H Stölting, A Stumpner (1998) Cell Tissue Res 294: 377>.

The sound processing found on the first level in the CNS shows the same basic principles which are described for different stations of various vertebrate systems:

- lateral inhibition for localization: local elements strengthen the directional difference in intensity and inhibit elements with axons to higher centres. In contrast to earlier findings, also in mammals lateral inhibition (and not delay lines) seems to be of major importance for directional processing <A Brand et al (2001) Nature 417: 543>

- sharpening of frequency tuning curves by frequency-specific inhibitions: certain types of neurons with axons ascending to higher centres receive rather broad excitation from a considerable part of the sensory cells; this excitation does not seem to be species-specific. Unidentified local elements (most likely DUM-type neurons) then reduce the frequency range by species-specific inhibition <e.g. A Stumpner (2002) J Comp Physiol A 188: 239>. Sharpening of frequency tuning was found in many vertebrates; pharmacological blocking experiments lead to similar results as compared to the bush crikket <e.g. H Zhang et al. (1999) J Comp Physiol A 184: 85>.

- the existence of various frequency channels: the current data indicate that auditory elements connecting the segmental ganglion of the ear with other parts of the CNS are tuned to a variety of frequencies which might allow the bush crickets a finer frequency discrimination as is known to date.

- a variety of types of temporal processing including rate dependence due to inhibition: response patterns and consequently responsiveness to different temporal patterns are

decisively influenced by inhibition as has been found in various vertebrates <e.g. QC Chen, PH-S Jen (2000) Hearing Res 150: 161>.

The advantage of the insect is the much easier accessibility of neurons, the identified-cell-approach and the possibility, to compare groups of related species on the level of single, identified neurons to understand potentially underlying evolutionary trends. In the ideal case specific functions can be attributed to single neurons by combined behavioural and neurophysiological experiments.

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306 Does Krill use bioluminescence for communication?

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Some is known about the light organs of krill: How they look like at the organ (Petersson G, 1968) and cellular level (Herring PJ & NA Locket, 1978), that they respond to serotonin in bath application with light emission (Kay RH, 1963). Apart from the major aim of our project, the neural, probably serotonergic control of the photophores, we try to find more information about the pathway which triggers the light output of this crustacean bioluminescence system: Utilization of these small, but highly developed organs by the animals for communication or another purpose has not yet been reported.

Captured animals of *Meganyctiphanes norvegica* (Northern Krill) did respond with more light production to artificial blue LED light flash stimuli (Fregin & Wiese 2001), and they also produce light when exposed to strong lights on-off gradient of white or blue light. Different intensities and different exposure times to the stimulus trigger varying durations and peak amplitudes of light production. Also, serotonin, forskolin and other substances injected directly into the animals are able to elicit or depress light production, and serotonergic immunoreactivity is present within the light organs (Fregin & Wiese, in prep.).

To find out more about the utilization of the photophores, we 3D-recorded the swimming behaviour and light production of *M. norvegica* kept in large tanks at Kristineberg Marine Biological Station, Sweden, in response to different stimuli. For the recordings two infrared cameras were synchronized. The signals were recorded simultaneously and the single pictures analyzed with Matlab6. The parallaxe between the two pictures was used to measure the z-coordinate. This technique is now established in our lab; the behavior of the animals in response to light flashes shows a seasonality, is best seen in spring.

Because we do not know if the animals produce light with their light organs spontaneously, we recorded the light production first in a water tank of ca. 400 l with >100 specimens over several days and secondly developed a deep sea probe with a photomultiplier to record the circadian bioluminescence patterns in the Gullmarfjord, Sweden at 0 to 100 m below sealevel. In the recordings we hope to find repetitive patterns e.g. flashes, which might be part of a communication channel, and which hopefully can be artificially mimicked (at least the time course) and elicit a response by captured animals.

In the tank, animal "dialogue" seems to build up without distinct time table, and the (preliminary) open water recordings show sparse flashing during the night, without peaks at dawn and dusk.

It has been reported in literature that light production in fish photophores within limits follows changes of illumination in the surround (Harper RD & JF Case, 1999). An apparatus has been build to measure light production in photophores absolutly independent from surrounding light.

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Intruder resident aggression in crickets — first insights into underlying mechanisms 307

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Animals in possession of a resource (e.g. food, females, territory) are generally highly aggressive towards intruding con-specific and usually succeed in defeating them. It is, however, poorly understood how resources influence the expression of aggression by the central nervous system. We describe an intruder-resident paradigm in crickets, which is highly stereotyped and thus allows insights into the elementary mechanisms underlying territoriality.

In the field, Mediterranean crickets compete for the acquisition of natural shelters such as gaps under stones or holes dug by other animals. We evaluated the influence of artificial shelters on aggression between male crickets in a small arena in the laboratory. Normally, losers of a previous aggressive interaction are submissive and avoid conspecific males for several hours after their defeat. However, losers become highly aggressive towards intruding winners immediately after residence in a shelter. No effect was observed by simply darkening the arena, or with shelters constructed of netting in a darkened arena, and illuminated shelters proved far less effective. Interestingly, loser aggression is positively correlated with duration of occupancy. Thus, after 2 min in the shelter losers still retreated from an opponent, but after 15 min residence, the aggressiveness of the losers was insignificantly different to that of naive males. Furthermore, the effect of shelter occupation on aggression is transient. Thus, losers offered a shelter for 15 min, and presented with an opponent 15 min after removing the shelter, were non-aggressive. Finally, the enhancing effect of shelter on aggression was no longer apparent in crickets specificly depleted of the amines octopamine and dopamine by α-methyl-tyrosine (AMT), but still effective in crickets specificly depleted of serotonin using α-methyl-tryptophan (AMTP). We speculate that the possession of a resource such as a shelter activates the octopaminergic system, which in turns enhances aggression.

308 Opponent assessment in aggressive encounters between crickets

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Dominance often provides access to limited resources. Agonistic behaviour is thus critically related to fitness. Aggressive encounters are, however, both risky and costly. Theoretical considerations accordingly emphasise the importance of information exchange while fighting, for animals to assess potential gains and loses. The biological mechanisms underlying the process of assessment are, however, poorly understood. By evaluating the effects of experimentally induced physical handicaps in a forced fight paradigm, our study reveals how informational cues are accumulated for the decision to fight or flee in crickets.

Surprisingly, crickets with either lamed mandibles, or blackened compound eyes (blinded), still exhibited the key elements typical of normal fighting behaviour. Furthermore, when matched against normal crickets, handicapped crickets do not win or loose significantly less than 50% of fights. However, blinded crickets win 91% of all fights against crickets with lamed mandibles. This suggests that in the absence of visual and physical cues from the opponent, a cricket persists longer at fighting and thus wins. Supporting this, we found that fights between pairs of crickets with the same handicap last longer. Compared with intact crickets, fights become progressively longer when progressively more organs are afflicted. Thus, whereas fights normally last only several seconds, fights between pairs of blinded crickets having both lamed mandibles and excised foreleg claws usually last 2-3 minutes.

Taken together, our data show that the persistance of aggressive behaviour by an individual depends only on the perceived actions of the opponent throughout a contest. Fights end when the summed sensory impact of that opponent exceeds some critical threshold for the decision to flee. Our findings are thus in accordance with predictions of the cumulative assessment hypothesis (Payne, Anim. Beh. 56: 651-662, 1998), rather than the more traditional concept of sequential assessment (Enquist & Leimar, J. Theor. Biol. 102: 387-410, 1983 and J. Theor. Biol. 127(2): 187-206, 1987) or war of attrition (Maynard Smith & Price, Nature 246: 15-18, 1973 and Maynard Smith & Parker, Anim. Beh. 24: 159-175, 1976). We aim now to investigate how opponent information is summed and integrated by the cricket nervous system for the desicion to flee.

Vibration sensitive interneurones of the primitive ensiferan (*Troglophilus neglectus*, Rhaphidophoridae) and their homology to acoustic interneurones of Ensifera

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Auditory tympanal organs of Ensifera have been shown to be serially homologous to and therefore evolutionary derived from vibration-sensitive chordotonal organs such as found in tibiae of meso- and metathoracic legs [Meier T., Reichert H. (1990) J. Neurobiol. 21, 592]. Since receptors of tympanal and atympanal tibial organs project to equivalent association areas of the segmental ganglia, it is generally assumed that they also make use of the same central network [Boyan G.S. (1993) J. Insect. Physiol. 39, 187]. To find a direct evidence for such a homologous organisation, we examined interneurones responding to foreleg vibration in a supposedly primitive deaf ensiferan *Troglophilus neglectus* (Rhaphidophoridae). We compared these neurones to the well examined acoustic neurones of Gryllidae and Tettigoniidae.

Sinusoidal vibrations of 50-5000 Hz and 2,25- 2250 cm/s^2 were applied to front legs of *T. neglectus*. In 65 animals a vibration-sensitive cell with the soma in the prothoracic ganglion was intracellulary recorded from and stained. Serial sections of the ganglia with stained cells were histologically examined. Nine descending neurones, six T-fibres, three ascending neurones and three local neurones of distinct morphologies were found. For several morphological types intraindividual physiological differences suggest coexistence of morphologically similar cells within the same hemi-ganglion. Several neurones resemble identified auditory neurones of Ensifera in their morphology. They correspond to DN1, DN4, TN1 and TN5 neurone of Gryllidae [Wohlers D.W, Huber F. (1982) J. Comp. Physiol 146: 161, Atkins G., Pollack G.S. (1987) J. comp. Neurol. 266: 389]. In *T. neglectus* these neurones occupy equivalent fibre tracts and association areas of the neuropile as their counterparts in Gryllidae [e.g. Wohlers D.W., Huber F. (1985) Cell Tissue Res 239: 555]. Only in "DN4" of *T. neglectus* an additional branch terminates within the vVAC, which is not found in DN4 of *T. oceanicus*. Physiological properties of *T. neglectus* neurones such as response type, latencies and presence of spontaneous activity are similar to those of cricket neurones [e.g. Atkins G., Pollack G.S. (1987) J. Comp. Physiol. A 161: 681].

Those cells described so far do not seem to be involved in intraspecific communication of crickets. Their tuning to low sound-frequencies, responsiveness to vibration and other stimuli such as wind and light and their low sensitivity to sound [Wohlers D.W, Huber F. (1982) J. Comp. Physiol 146: 161, Atkins G., Pollack G.S. (1987) J. Comp. Physiol.161, Kühne R., et al., (1984): J. Insect. Physiol. 30, 575] suggest that these units are part of a predator-detecting system mediating escape behaviour. We assume that vibration-sensitive interneurones described in *T. neglectus* serve for the same function. In agreement with this is the very similar neuroanatomy of the *T. neglectus* neurones with their likely homologous partners in Gryllidae, indicating that no dramatic changes of this system have occurred in the course of evolution. No neurones morphologically similar to those having a crucial role in auditory processing in Ensifera, like local Omega cells

and ascenders, have been found among vibration sensitive neurones of *T. neglectus*. For the search of the homologous neurones to these exclusively acoustic cells, in non-hearing species applications of other techniques (such as immunohistology) are currently applied.

In conclusion, our data indicate that only a part of the auditory network of Gryllidae is homologous to the vibration-sensitive central network connected to the tibial organ of *T. neglectus*. The typical auditory neurones of crickets might have been recruited from some other sensory system, preadapted for auditory processing.

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310 The vibratory interneurons in the central ganglion of the southern green stinkbug *Nezara viridula* (L.) (Heteroptera: Pentatomidae)*

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Vibrational communication is an important part of mating behavior of the southern green stinkbug *Nezara viridula* (L.). On a large scale it enables male vibrational directionality and during courtship species specific songs take part in species recognition [1].

N. viridula uses plants as the medium for vibrational communication. Frequencies around 100 Hz are transmitted through the plant tissue with lowest attenuation and that coincides well with the frequency spectra of *N. viridula* songs. The dominant frequency component of the female calling song lies between 80 and 140 Hz [2] and higher frequencies are well expressed. Insects use bending waves for communication through the substrate [3]. During transmission through the plant tissue the signal is altered due to the nature of the bending and standing waves. The aim of our study was to investigate how these changes are coded within the central nervous system.

N. viridula detects substrate vibrations with chordotonal organs situated in all six legs. Most sensitive vibroreceptor involved in vibrational communication is the subgenual organ [4]. In species *N. viridula* it consists of two scolopidia, each with only one receptor cell [5]. Cokl and Amon [6] described four functional types of vibratory interneurons which have different frequency and intensity sensitivity. All of the described interneurons responded to the stimuli by excitation.

In our study we recorded extracellular responses to vibratory stimuli of 316 interneurons in the central ganglion of *N. viridula*. We divided the interneurons into three groups according to the nature of their response to the stimuli (excitation, inhibition or both). This rough division was based on responses to 100 ms long stimuli which frequency ranged from 50 to 4000 Hz; the acceleration level was 100 cm/s^2. Within each group we described different types of interneurons according to the shape of their threshold curves. We found three types of interneurons that responded with inhibition and four types

that responded with excitation. In the group of interneurons, which responded excitatory and inhibitory we found two types. The first responded with excitation to lower and with inhibition to higher frequencies and the second type responded vice-versa. We present the functional properties of the nine described types that differed in their frequency and intensity sensitivity, the latency of the response and the type of response (tonic, phasic).

Although the subgenual organ of *N. viridula* consists of only two receptor cells, a great variety of interneurons in the central nervous system enables the male to resolve the discrete changes that occur while the signal is being transmitted through the plant tissue and eventually locate the singing female.

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Homologues of the motor protein prestin in lower vertebrates and insects

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Prestin, one of nine members of the anion transporter family SLC26, is the outer hair cell molecular motor responsible for active mechanical amplification in the mammalian cochlea. Active amplification is present in a variety of other auditory systems, yet the prevailing view is that prestin is a motor molecule unique to mammalian ears. Here, we identify further prestin-related SLC26 proteins that are expressed in the auditory organs of lower vertebrates and insects. Sequence comparisons revealed the presence of SLC26 proteins in mosquitoes (Anopheles, agCP10636 and agCP7199), flies (*Drosophila*, CG5485), and fish (Danio, z06s031971; Anguilla, BAC16761). The fly homologue was cloned and, using *in situ* hybridization, shown to be expressed in the fly?s auditory organ. In mosquitoes and zebrafish, in turn, the expression of prestin homologues was demonstrated for the auditory organ using highly specific riboprobes against rat prestin. We conclude that prestin-related SLC26 proteins are widespread, possibly ancestral constituents of auditory organs and thus, likely to serve salient auditory roles in mammals and across taxa.

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312 Electrophysiological Characterisation of Hair Cells from the Hearing Organ of the Zebrafish (*Danio rerio*) reveals two different Types of Potassium Currents

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As the zebrafish (*Danio rerio*) is becoming an increasingly important model for the vertebrate genome its well developed sense of hearing (up to frequencies of 2.5 kHz) makes it suited for identifying new genes

involved in hearing. To establish electrophysiological data for wild type animals we studied the voltage-activated currents in hair cells from the lagena, the hearing organ of the zebrafish. Whole cell currents were recorded from hair cells at the edges of explants of the lagena since isolating single hair cells would render them leaky. Whole cell recordings (outside solution: 145 mM NaCl, 3 mM KCl, 10 mM CaCl2, 10 mM HEPES, 310 mosm* kg^{-1}, pH 7.2; internal solution: 20 mM KCl, 120 mM K-gluconate, 0.3 mM GTP, 10 mM Na-phosphocreatine, 10 mM EGTA, 4 mM Na2-ATP, 2 mM MgCl2, 10 mM HEPES, 290 mosm* kg^{-1}, pH 7.2) revealed voltage-activated outward currents that could be identified as potassium currents. Two types of cells could be distinguished due to differences in the kinetics of current activation and inactivation. One cell type showed a current that activated transiently at negative membrane potentials (about -58 mV) and inactivated rapidly, which was classified as I_A. The other type of hair cells, which was lacking the I_A current, showed a slowly inactivating current (I_K) that was activated at membrane potentials positive to -27 mV. The two types of cells also differed in their peak K^+ conductances (I_A cells: 8 nS, I_K cells: 19.5 nS) and their capacities (I_A cells 1.6 pF, I_K cells 2.8 pF). Mean current densities were 3.4 nS/pF (I_A cells) and 7.3 nS/pF (I_K cells). A pharmacological identification of the two groups of K^+ channels could not be accomplished due to the short lifetime of patch recordings (3 min). We aim at optimisation of preparation conditions and solutions to ensure recording time spans of several minutes as in higher vertebrate hair cells.

313 Neuronal encoding of ultrasonic stimulation in a fish

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Many species of odontocete cetaceans (toothed whales) use high frequency clicks (60-170 kHz) to identify objects in their environment, including potential food fish (1). Behavioral studies have shown that American shad, *Alosa sapidissima*, can detect ultrasonic signals similar to those of their odontocete predators, show strong escape behavior in response to ultrasonic pulses between 70 and 110 kHz, and can determine the location of the sound source at least in the horizontal plane (2,3). The present study examines ultrasound detection by American shad and provides the first insights into the neuronal encoding of ultrasound signals in any non-mammalian vertebrate. Ultrasonic

responsive units could be subdivided into three types, broadband = 40 kHz bandwidth, narrowband = 30 kHz bandwidth, and dual band units (e.g. responses to 20-30 kHz and 70-90 kHz but no response in between). Units were recorded at various depths (110 µm to 4510 µm). Lesions of recording sites were found in different nuclei of the rombencephalon. Most were in the dorsal nucleus, the eminentia granularis (EG), with fewer in then the secondary octaval population (SO), and fewest in the descending octaval nucleus (DON). The background activity of peripheral ultrasound units varied between 140 AP/s (action potentials/s) (± 9.8) and 0.08 AP/s (± 0.1) (average: 20.5 AP/s (± 18 AP/s), median: 9.1 AP/s); sonic units recorded for control purpose in the same areas showed baseline activity between 50 and 75 AP/s). The response dynamic to ultrasound stimuli varied from phasic-tonic responses (excitatory or inhibitory) to sharp phasic responses. All but two units responded only to ultrasound stimulation. Ultrasound units did not phase couple to any stimulus frequency. Units recorded in the eminentia granularis resembled responses of constant latency neurons found in the VNLLc of bats. We suggest that the ultrasound pathway in *Alosa* is independent from the sonic pathway and that this species might process azimuthal directional ultrasound information by means of Interaural Time Delays (ITDs). The ultrasonic pathway in *Alosa* appears to be a feature detector that is adapted by some mechanisms (e.g., frequency, intensity) to the odontocete echolocation signals.

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Effects of BAPTA in Scala media on the spectra of lizard spontaneous otoacoustic emissions. 314

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In land vertebrates, active processes in the inner ear act to enhance hearing sensitivity and frequency selectivity. In the Australian bobtail lizard, spontaneous otoacoustic emissions (faint sounds produced by the ear) are generated by an active mechanism integral to the hair-cell bundle (Manley, G.A., Kirk, D.L., Köppl, C., Yates, G.K., 2001, PNAS (USA) 98, 2826-2831). Calcium ions are thought to be intimately involved in active processes driving hair-cell stereovillar bundles (Hudspeth, A.J., 1997 Curr. Opin. Neurobiol. 7:480-486). We have used iontophoresis of the calcium chelator BAPTA into the Scala media of anaesthetised Bobtail lizards to reduce the level of calcium in the endolymph *in vivo*, while simultaneously monitoring spontaneous otoacoustic emission (SOAE) spectra.

Since the negative DC currents used to iontophorese themselves affect SOAE directly (Manley G.A. and Kirk, D.L., 2002, JARO 3, 200-208), in control experiments we compared BAPTA effects to those of control current applied through pipettes filled only with KCl. Iontophoresis of BAPTA (to an estimated peak level of 5mM in the endo-

lymph) resulted in much larger, more prolonged downward shifts in the frequency of SOAE peaks than when injecting current alone, and these shifts took much longer to recover. Full recovery of the spectral peaks took more than one hour, presumably due to slow clearance of BAPTA from Scala media. A similar fall in oscillation frequency has been observed in bundle oscillations *in vitro* in the bullfrog sacculus (Martin, P. & Hudspeth, A. J., 1999, PNAS (USA) 96, 14306-11).

The availability of calcium ions has more than one influence on hair-cell bundles. It can influence both mechanisms involved in active processes and mechanisms driving the hair-cell adaptation system. Reducing calcium can affect oscillation frequencies by reducing the rate of hair-cell adaptation and by increasing the amount of time the bundle spends in the negative-stiffness region. The present data do not select between these possibilities. They do, however, strongly implicate calcium ions in some mechanisms involved in the generation and control of active motility in the inner ears of non-mammals.

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315 6-OH-Dopamine lesions in anuran amphibians

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To investigate the effects of dopamine depletion on acoustically guided behavior of anurans, we conducted phonotaxis experiments with female gray treefrogs (*Hyla versicolor*) before and 90 minutes after bilateral injections of solvent, 3 µg, 6 µg, or 12 µg 6-hydroxydopamine (6-OHDA) into the telencephalic ventricles. In experiments with one loudspeaker playing back a standard artificial mating call we analyzed the effects of 6-OHDA on number of phonotactic responses and response time (motor effects). Aditionally, we calculated a "phonotaxis score" for each female, which is defined as the ratio of her mean total response time before injections to her mean total response time after injections. In choice tests with two loudspeakers we measured the degree of distraction from the standard call (20 pulses/s) by three different variants with altered pulse-rate (30/s, 40/s, and 60/s). We calculated a "choice score" for each female before and after injections, defined as the ratio of the mean response time in the one-speaker experiment to the response time in the choice situation. Five days after experiments, brains were fixed and stained with an antibody against tyrosine hydroxylase. Number of labeled neurons were counted in the suprachiasmatic nucleus, posterior tuberculum, interpeduncular nucleus, and locus coeruleus. Neuronal counts were normalized and correlated with behavioral scores.

6-OHDA lesions (6 µg and 12 µg) led to increased response times and lower phonotaxis scores (compared to controls) in one-speaker tests, mainly due to longer immobile periods before the animals started movement. In choice tests females showed after 6-OHDA injections lower choice scores than before, while control animals reached higher choice scores after vehicle injections. This difference was statistically significant for

stimulus pair 20/s versus 60/s. Thus, females showed a higher distraction from the standard stimulus by the most irrelevant stimulus (60/s) compared to sham injected controls. This might be caused by impaired filtering of irrelevant information, which also occurs in Parkinson patients.

Immunohistochemical analyses revealed a loss of catecholaminergic neurons in the suprachiasmatic nucleus, posterior tuberculum, and interpeduncular nucleus after injection of 6 µg and 12 µg 6-OHDA, but not in the locus coeruleus. The normalized number of immunoreactive neurons in the posterior tuberculum was negatively correlated with total response time in the one-speaker test, and positively correlated with phonotaxis scores. We conclude that bilateral 6-OHDA lesions in anuran amphibians are a suitable model for Parkinson's disease, since anurans suffer motor (difficulty to start movements) as well as cognitive symptoms (impaired filtering of irrelevant information).

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Directional characteristics of auditory nerve fibers in the gray tree frog, *Hyla versicolor*. 316

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Behavioral studies of directional hearing in terrestrial frogs have been concentrated on the hylid tree frogs, where females show a robust phonotactic response to the conspecific mating call. It has been shown that female *H. versicolor* and other hylids locate conspecific calls with an average error angle of approximately 20 degrees(see, e.g. Jørgensen and Gerhardt, JCP A 169: 177-183, 1991). Biophysical studies of *H. versicolor* have shown that the eardrum directivity around the call frequencies is largely caused by acoustical coupling of the eardrums through the wide Eustachian tubes and mouth cavity, leading to pressure-difference receiver directivity with ovoidal characteristics and ipsi-contralateral differences of up to 10 dB (Jørgensen and Gerhardt, *op.cit.*).

Neurophysiological studies of directionality in frogs have shown that the high-frequency directionality of the auditory nerve is comparable in shape and magnitude to the eardrum directionality, but that low-frequency directional information is encoded by unknown, non-tympanic pathways (Feng, JASA 68:1107-1114, 1980, Jørgensen and Christensen-Dalsgaard, JCP A 180:493-502, 1997). However, the neurophysiological studies have been done only on larger ranid frogs that do not show a very robust phonotactic behavior, and where, arguably, there is a small selection pressure for phonotaxis towards the mating call. In comparison, the neural processing in hylid tree frogs could be enhanced due to the importance of phonotaxis in these species.

Here, I report on directional characteristics of single auditory nerve fibers in gray tree frogs. The auditory nerve was exposed using a dorsal approach and the frog was placed in a free field setup and stimulated by sound (tone bursts, amplitude-modulated tones and noise, and sampled H. versicolor calls) from twelve speakers. Results from 89 fibers show an ovoidal directional characteristic at high frequencies (above 800 Hz) and either ovoidal or figure-eight characteristic at low frequencies. The fibers show maximal re-

sponses from ipsilateral-caudal angles and minimal responses from contralateral-frontal angles. While the results are qualitatively similar to the earlier studies in ranid frogs, the directionality is larger, probably reflecting specialization for directional hearing in gray tree frogs. The origin of this increased directionality is unknown.

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317 The origin of directional sensitivity in low frequency auditory nerve fibers in the grass frog, *Rana temporaria.*

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Low-frequency auditory fibers in the frog show a pronounced sensitivity and directionality at frequencies from 100 to 400 Hz, where the directionality generated by the tympanic pressure-difference receiver is insignificant. The origin of this extratympanic sound sensitivity is unknown, and pathways through the substrate and through the operculum has been ruled out recently. Jørgensen and Christensen-Dalsgaard (1997) speculated that sound-induced vibrations of the skull might stimulate the low-frequency auditory fibers directly. If this is the case, the directional characteristics of the fibers should reflect a differential sensitivity to vibration stimulation of the skull from different directions. Therefore, we have started an investigation of the sensitivity of auditory- and vibration-sensitive fibers to three-dimensional vibration.

The 8th nerve of adult grass frogs was exposed using a dorsal approach. Single-unit responses were recorded with glass microelectrodes (20-40 MW). The frog was stimulated with vibrations from 3 directions (X: cranial-caudal; Y: contralateral-ipsilateral; Z: dorsal-ventral) using three orthogonal Brüel & Kjær (B&K) vibration exciters and sound using a closed coupler sealed over the ipsilateral eardrum. The stimulation and recording was performed by a Tucker-Davis Technologies (TDT) system and controlled by a PC using custom-made software. The system was calibrated prior to every series of experiments using a 3D-accelerometer, mounted directly above the frog's head. The sound was calibrated with a B&K microphone mounted in the coupler.

We found three types of neurons: Neurons that were excited only by vibration, neurons that were excited by sound and by vibration and neurons (usually with a high BF) that only responded to sound.

The neurons that were excited by vibration alone had their best sensitivity in the range between 40-80 Hz. The neurons that were excited by sound and vibration usually had the highest sensitivity in the area between 160-200 Hz. Most of the vibration sensitive neurons had the best sensitivity on the Z-axis, of the remaining neurons the main part was most sensitive along the X-axis and only a few had the highest sensitivity along the Y-axis. The X-axis sensitivity accords well with the figure-eight directivity with a frontal null shown by the low-frequency auditory fibers to free field sound. The saccular macula is oriented in the vertical plane, and therefore the saccular vibration sensitive

fibers would be assumed to be most sensitive to stimulation along the Z-axis. This sensitivity could be useful in detecting ground vibrations generated by predators.

Echoes of bat-pollinated bell-shaped flowers: Conspicuous for nectar-feeding bats? 318

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Nectar-feeding glossophagine bats are able to find bat-pollinated flowers even in the highly cluttered environment of neotropical rainforests. To detect and recognize these small and scattered nectar sources in this habitat they rely on olfactory cues and vision but –like all microchiropteran bats– largely on echolocation, too. Zoophilous plants generally match their floral traits to the sensory characteristics of their animal pollinators in order to ease detection. Chiropterophilous plants depending on echolocating bats as pollinators may therefore be expected to have evolved flowers with acoustically conspicuous echoes, which facilitate detection and recognition. As it is poorly understood how bats manage to find and recognize flowers acoustically, we investigated the flower echoes of some plant species pollinated by bats.

In order to obtain the echoes actually arriving at the bat, they were measured with a small loudspeaker and a microphone whose size and relative position resembled a bat's head.

Echoes of bell-shaped bat-pollinated flowers showed characteristic features with respect to the echoes they reflected to a calling bat, making them different from the echoes of leaves or other objects in their surroundings: the echoes were comparatively long and of complex "coloured" spectral composition. Both features resulted from their bell-shape. Duration was increased in proportion to the depth of the bell because the sound needed more time to travel in and out of the bell and multiple reflections further prolonged the echo. The characteristic "coloured" spectral composition of the echo originated from interference due to multi-path propagation within the bell, enhancing some frequencies and canceling others. For comparison we investigated echoes of simple concave forms (hollow sphere and paraboloid); they showed the same characteristic features (increased duration and "coloured" spectral composition).

Owing to the specific shape of the flowers and the artificial forms, characteristic "spectral directional patterns" resulted when the spectra of the echoes were plotted against the angle of sound incidence.

We suggest that bats are able to recognize such flowers - and probably other objects as well - not only by the characteristic spectral composition of a single echo but additionally by comparing sequential echoes, at the same time taking into account their exact calling position relative to the object.

319 Echolocation range and wing beat period match in aerial hawking bats

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Aerial-hawking bats face the problem that both flight and echolocation exert independent and possibly conflicting influence on their call intervals: On the one hand, these bats couple call emission to their wing beat in order to reduce the costs for the production of their intense echolocation calls. That way, flight morphology and flight style ultimately determine their mean call intervals and thereby the time window in which these bats can listen for echoes without risking call-echo overlap or the increased effort for assigning each echo to the call it originates from. Owing to the constant propagation speed of sound in air this time window corresponds to the distance range of available objects. On the other hand, aerial-hawking bats searching almost empty skies for prey by echolocation can only exploit their full echolocation range unambiguously if they call again after the echo from the outer limit of their echolocation range would have arrived. We compared wing beat period to the delay of this last expected echo for different types of objects.

Acoustic Flight Path Tracking was employed to measure source levels of echolocation calls of 11 European aerial-hawking bat species in the field. Source levels were very high extending the known range to 133 dB peSPL. Even the smallest European bat produced up to 128 dB peSPL. From these source levels we calculated maximum detection distances for insects and larger objects with spherical spreading of the echo (e.g. conspecifics, flying predators) as well as for large background targets mirroring the echo back to the bat (e.g. water surfaces, walls). Wing beat periods were derived from call intervals.

Heavier bats generally achieved longer detection ranges and detection range increased with the target strength of the insect (5 m and 25 m for the largest insects; between 2 m and 7 m for the tiny culicids). Yet, for background targets, much higher detection ranges were derived (13 m to 90 m).

In all species all detection ranges for insects lay well spread within the overlap free window set by the wing beat period, making more or less full use of the available time window. Furthermore, for small and medium sized bats there was a tight matching between wing beat and detection range for the largest objects with spherical spreading. Maximum echo delays for background targets were much longer than wing beat period in all species. Yet, the tendency to skip or individually mark successive calls as a countermeasure correlated with the species' detection range for background targets.

We conclude that aerial-hawking bats searching for flying prey in fact match their maximum detection range for flying targets to their wing beat period. By doing so they take advantage of efficient call production and at the same time scan the skies without risking call-echo overlap or assignment problems and also without wasting time by waiting too long.

We argue that a species' search call frequency is just as high that the resulting detection range matches their wing beat period.

Discrimination of rotary hollow forms by echolocation in the nectar-feeding bat *Glossophaga soricina* 320

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Since the pioneering work of Ronald Griffin a large number of studies investigated how insectivorous bats can detect and locate flying prey. However, data on object recognition based on echolocation is scarce, and it is not well understood, how motionless objects can be recognized by echolocation, in particular in clutter-rich surroundings.

Nectar-feeding bats like *Glossophaga soricina*, on their foraging flights, perform several hundred visits to many different flowers in one night, and are able to quickly aquire new nectar sources, which they find guided by visual and olfactory cues and by echolocation. In the absence of the two first they still manage to detect flowers using their echolocation system alone. Delivering more than thousand decisions per night they are well suited for training experiments testing object discrimination based on echolocation.

In a first set of experiments we asked whether and how well *Glossophaga soricina* can learn to discriminate various hollow forms (36 mm diameter). Training followed a „two alternative forced choice" paradigm, which was controlled by a computer that registered the visits of the bat, released a reward for a correct decision and exchanged the position of the positive and negative form in a random order. The bats easily learned not only to discriminate various hollow forms, such as paraboloids of different curvature and depth, but also forms of the same diameter and depth differing only with respect to their curvature. The echoes of such forms are similar with respect to their duration and intensity but different in their spectral pattern composition, which, for each form, changes in a characteristic manner in dependence of sound incidence angle. We suggest that *Glossophaga* identifies forms by their specific acoustic image (the sequence of echoes relative to the bats position) perceived during approach.

In a second experiment we investigated how well bats can discriminate between forms of the same shape presented at different sizes. In a first step the bat was trained to be rewarded at a hollow hemisphere of a given diameter (eg. 38 mm). In succeeding tests the bat was simultaneously confronted with a set of hollowspheres mounted at eight protruding spokes of a slowly back and forth driving wheel. We found that spheres differing in radius by ± 1 - 2 mm were significantly less frequently visited than the (in the test situation) unrewarded positive sphere. In these experiments, for discrimination, the bats could have used differences in size and depth also, but it is more likely that they learned the absolute pitch of spectral bands, which drastically varied with changing radius of the sphere.

321 Temporal response patterns of cat single auditory nerve fibers with simultaneous electric and acoustic stimulation (EAS)

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Patients with residual hearing in low frequencies and a profound hearing loss in the high frequency range often have insufficient speech comprehension with conventional hearing aids. Electrical stimulation of the non functional high frequency region by an cochlear implant in addition to an acoustical stimulation of the low frequencies by a hearing aid may improve speech perception (v.Ilberg et al 1999). In a previous study auditory nerve fiber responses of normal hearing cats to combined electrical and acoustical stimulation were studied with respect to spectral response properties (ARO Abstr.2001, 908). Response areas didn´t show dramatic changes according to characteristic frequency, threshold and bandwidth with a combined stimulation. The present study is focused on the question whether simple temporal response patterns evoked by electrical stimulation are influenced by a simultaneously applied acoustical stimulus and vice versa.

Acute experiments were performed in adult normal hearing cats implanted with round window electrodes used for extracochlear electrical stimulation. A 125 Hz sinusoid served as electrical stimulus. For acoustic stimuli tone-bursts of 50-100 ms duration and different frequencies and intensities were used. After determination of the CF of a fiber a combination of acoustical tone bursts at CF and electrical sinusoids (phase locked to acoustic burst onset) 6dB above single fiber threshold served as stimuli. The PSTH, interval histogram and synchronization index (SI) were calculated for each EAS response and compared to responses evoked by single stimulation condition.

Evaluation of SIs generally suggests a slight to prominent decrease of synchronization to the electrical sinusoid with EAS. While in most cases an electrically evoked response still exists with EAS some fibers showed a total suppression of the electrical response by the acoustic stimulus. Switching off the acoustical stimulus led to a highly synchronized response pattern to electrical stimulation. Synchronization to the acoustical stimulus of phase-locking fibers was less deteriorated by the electrical stimulus. These results suggest that the acoustic response is less affected by the electrical stimulus than vice versa. The activity of hair cells may play the dominant role in generating the EAS responses.

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Comparison of auditory threshold curves measured with otoacoustic emissions and evoked cochlear potentials in the gerbil 322

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The noninvasive measurement of Distortion-product otoacoustic emissions (DPOAEs) has become an important tool in evaluating mechanical cochlear sensitivity both for clinical purposes and in basic research. DPOAEs are mechanical vibrations produced by nonlinear amplification of low level sound by outer hair cells. They travel back through the middle ear and can be recorded at the ear drum.

As experimental animals we used gerbils, since they have a wide hearing range that extends from frequencies below 0.5 kHz to about 50 kHz. Therefore this species is an excellent model to study peripheral auditory processing both for low frequencies as they are relevant for human hearing and for ultrasonic frequencies.

During stimulation with two tones we simultaneously measured acoustic distortion products at the ear drum and their electrical correlate in the form of cochlear microphonic potentials (summed outer hair cell receptor potentials) at the round window of the cochlea. In addition we determined cochlear microphonic and auditory nerve sensitivity during single tone stimulation.

While there is a very good correspondence of the different threshold measures for frequencies above 4 kHz, at lower frequencies the acoustic DPOAE thresholds are significantly less sensitive than the physiological threshold measurements. Both a reduction of outer hair cell amplification at low frequencies and/or the presence of multiple distortion-product sources in the cochlea could be involved in the threshold discrepancies. Therefore, differences in auditory processing of low versus high frequencies should be taken into account when assessing cochlear sensitivity by noninvasive measurement of otoacoustic emissions.

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Cochlear sensitivity in the lesser spear-nosed bat, *Phyllostomus discolor* 323

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Behavioral auditory thresholds of *Phyllostomus discolor* are characterized by two sensitive regions separated by an insensitivity at 55 kHz. To investigate if these characteristics are due to cochlear properties, we recorded DPOAE thresholds to make conclusions about cochlear sensitivity. DPOAEs are generated in the cochlea due to active nonlinear mechanical amplification of low level sound signals by outer hair cells. They can be registered by a coupler system, incorporating a microphone and two loudspeakers near the tympanum; which is an efficient noninvasive method to invastigate cochlear mechanics. To reduce the risk of injury of the tympanic membrane, the animals were

lightly anesthetized by Isoflurane. We found that the sequence of a threshold insensitivity at 55 kHz followed by a sensitive minimum at about 85 kHz can not be confirmed in DPOAE measurements: the DPOAE-threshold curves have an absolute minimum at approximately 30 kHz and at higher frequencies the threshold continously increases lacking any extreme sensitivities. Therefore either features of the outer ear or central neuronal processing seems to be responsible for the threshold minimum and maximum at higher frequencies seen in behavioural threshold measurements. Additionally we tested if the used anesthetic, Isoflurane, has an influence on cochlear properties: In first experiments comparing awake and anesthetized animals we found that Isoflurane enhances the DPOAE amplitude at stimulus levels clearly above threshold, which could be due to an influence of the anaesthetic on the efferent olivocochlear system.

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324 Noise Trauma in the 129/S4 Mouse, a Strain with "Tough" Ears

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There is a large variation in the susceptibility to acoustic trauma in different mammalian species, but also in different strains within a single species. Especially the many different mouse strains differ in their susceptibility to age- and trauma- related hearing loss as well as in their hearing sensitivity. The group of 129/Sv strains, which is often used to produce knockout mutants seems to be especially resistant to sound trauma. One of its mutants (129/SvEv) has been shown to be less sensitive to sound and very resistant to hearing loss induced by a 8 - 16 kHz noise band (Yoshida et al., 2000). We investigated what level and duration of sound exposure is necessary to cause acoustic injury, that leads to a permanent threshold shift (PTS) in one of the 129/Sv mice strains (129/S4). Control auditory brain stem responses (ABR) of 46 mice were measured at the age of 4-6 weeks. Two silver wire electrodes were placed subcutaneously rostral to the pinna on both sides. A loud speaker was placed in the external ear canal. Gaussian shaped tone pips (2/3 octave bandwidth) were used to determine ABR-response thresholds at 7 frequencies from 1-64 kHz in general anesthesia. For acoustic trauma, unanesthetized mice were placed in a reverberation chamber and stimulated with octave band noise of 110 dB SPL, or pure tones of up to 120 dB SPL and different exposure times and frequencies. After acoustic overstimulation, ABR-audiograms were recorded weekly, up to 4-6 weeks after sound trauma. The remaining PTS after sound trauma was correlated with the morphology of the mouse ear, using scanning electron microscopy of whole cochleae.

We found the following: In 129/S4 mice pure tone stimulation in the frequency range from 6 to 16 kHz of up to 120 dB SPL did not cause any permanent hearing loss. The most effective center frequency of octave band noise in causing substantial permanent hearing loss was 11.3 kHz (5.66kHz, 8kHz, and 11.3kHz were used). Exposure with octave band noise for five days at levels of 110 dB SPL did not cause significantly more threshold shift than exposure for three days. From the parameters used, octave band

noise stimulation with 11.3 kHz center frequency and exposure time of three days was the most effective to induce permanent threshold shift in 129/S4 mice (about 40 dB maximum). However, even the largest threshold shift was correlated with minor morphology changes only, at least in those inner ear structures that are accessible via scanning electron microscopy, e.g. only the inner row of outer hair cells was affected. The results show, that 129/S4 mice are very resistant to noise trauma compared to CBA/J or C57/BL6 mice and indicate that 129/S4 mice are not suitable animal models for noise induced hearing loss.

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Late maturation of hair-cell bundle morphology in the auditory papilla of the barn owl

325

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Among birds, the barn owl (*Tyto alba*) is a well-known auditory specialist, using acoustic cues to localize its prey at night. An overrepresentation of behaviourally-relevant frequencies is already seen at the level of the hearing organ, the basilar papilla. Correlated with this and superimposed on the typical avian pattern, the morphology of the sensory hair-cell bundles is unusually uniform in the specialized regions.

Owls are altricial birds, hatching in an immature state with very restricted sensory capabilities. Auditory brainstem and midbrain centres in the barn owl continue to develop for months posthatching. However, the time course of papillar development is currently unknown. Here we report the posthatching maturation of hair-cell bundle morphology in the young barn owl's basilar papilla. Specifically, the number of stereovilli per bundle and the orientation of the bundles' mechanosensitive axes were investigated. Both parameters are presumed to be crucial for adult-like hair-cell function.

Basilar papillae of barn owls aged between P5 and P60 were prepared for SEM using standard methods. Samples of hair cells were analyzed at up to 9 equidistant positions along each papilla. In the first week posthatching, the stereovillar bundles were clearly immature, with the shortest stereovillar row still grading into adjacent microvilli. Resorption of these supernumerary villi was in progress by P11 and was finished by approximately the end of the third week. Correlated with this, the pattern of stereovillar numbers both along and across the papilla appeared to be mature. In contrast, the adult orientation of the stereovillar bundles was only achieved significantly later. Within the first month posthatching, medial hair cells of the papillar apex gradually deviated more and more from a strictly radial orientation of their mechanosensitive axis. However, even at P32, the adult orientation had not been reached. This was only seen in our next-oldest specimen, at P60.

Compared with the precocial chicken, hair-cell development in the barn owl followed the same pattern, but is clearly prolonged. Adult conditions were not reached within the first month posthatching and could possibly take as long as two months.

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326 Implication of FGFs during induction and morphogenesis of the inner ear

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The inner ear is a sensory organ responsible for the senses of hearing, balance and detection of acceleration in vertebrates. It develops from the otic vesicle, a transient structure that is formed by invagination of a placode, which arises from the ectoderm next to the hindbrain at the level of rhombomeres 5 and 6 during early stages of the development. Next, the otic vesicle undergoes various processes including morphogenesis, proliferation and differentiation giving rise to the mature inner ear. The formation of the otocyst is dependent on inducing signals coming from the endoderm, mesoderm and neuroectoderm. In those processes different members of the FGF (fibroblast growth factor) gene family have been implicated.

By using loss- and gain-of-function approaches carried out in avians and mammals we report our findings on the implication of FGFs during the induction in the inner ear. We have performed *in ovo* electroporation of various FGFs into the neural tube which resulted in structures with otic fate. To analyse the consequences of a loss of FGF function we have started to electroporate antisense morpholinos in chicken embryos. To clarify the roles of FGFs during inner ear development in mammals we are analysing various transgenic mouse lines which ectopically express FGF genes.

327 Distortion Product Otoacoustic Emissions Do Show Different Growth Behaviour In Guinea Pigs With Middle Ear And Inner Ear Dysfunction

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Distortion Product Otoacoustic Emissions Input/Output-functions (DPOAE I/O) give an insight into the compressive, non-linear sound processing of the cochlea. With an inner ear dysfunction a steeper I/O-function is observed, which can be interpreted as loss of sensitivity and compression of the cochlear amplifier (Mills et al. 1996, Janssen et al. 1998, Kummer et al. 1998). Due to the linear sound processing of the middle ear, one can assume that the DPOAE growth behaviour remains unaltered with a sound conduc-

tion dysfunction. If that were true, a differentiation between middle ear and inner ear dysfunction would be possible based on DPOAE measurements. To test that, dysfunctions of the middle and inner ear of Guinea pigs were induced and DPOAE I/O-functions recorded. All experiments were carried out under anaesthesia. Physiological saline solution (0.1 ml) was applied into the opened bulla of the ears of group A to induce sound conduction dysfunction. Subjects of group B were exposed to loud broadband noise (L = 115 dB once for 2.5 h on 2 consecutive days) to induce a cochlear dysfunction. DPOAE I/O-functions were recorded before and after the manipulations at the following f2 frequencies: 8.0, 6.7, 3.3, 2.8, 2.3, 2.0 kHz (f2/f1 = 1.2). Primary tone levels were set after an especially developed scissor paradigm for Guinea pigs (L1 = 0.46 L2 + 41 dB SPL, 15 dB SPL < L2 < 60 dB SPL, Michaelis et al., 2003). The middle ear dysfunction results in the expected reduction of the DPOAE amplitude independent of primary tone level. The cochlear dysfunction verifies the already known reduction of DPOAE amplitude dependent of primary tone level. In contrast to the cochlear dysfunction, where a loss of compression can be observed, the growth behaviour of the middle ear dysfunction shows no loss of compression. Thus, DPOAE I/O-functions allow a differentiation between middle ear and inner ear dysfunction. Further studies will have to show the usability of this method for future clinical diagnostics.

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The motion of the subtectorial space and its resulting fluid motion in the guinea pig cochlea 328

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Shearing motion between the lower surface of the tectorial membrane (TM) and the reticula lamina (RL) produces fluid motion in the subtectorial space of the organ of Corti (CO). Using vibration measurements of the transverse motion of these surfaces, we provide evidence for stimulus evoked changes of this space.

The results derive from an in-vitro preparation of the third turn of 17 guinea-pig cochleae where the presumed characteristic frequencies were in the range 0.9 - 1.2 kHz. All other turns of the cochlea were removed. Reissner´s membrane was intact over this turn, enabling better preservation of the TM. The vibration measurements were in response to intra-cochlear electrical stimulation. For stimulation, two platinum electrodes and one gold electrode were used, the latter was also used as a mirror. The stimulus was a multi-tone signal consisting of 81 frequencies, from 480 Hz to 70 kHz, with equal amplitude but random phase. The electrically-induced vibration pattern of the CO was measured throughout its depth, using a laser Doppler vibrometer (Polytec OFV 302). With a depth resolution of ±1.8 µm from the focus plane (at -10 dB), the measurements on different anatomical levels - RL and upper and lower sides of the TM - did not contribute to each other considerably.

The RL vibration pattern reveals a significant phase shift of 180 degree between the inner (IHCs) and outer hair cells (OHCs) and also between the OHCs and Hensen´s cells. A phase shift was not found on the TM, the motion of which was in phase with that of the RL at the OHCs. The RL vibration amplitude above the OHCs (9.8±4.3 nm/mV) was, on average, three times larger than that above the IHCs (3.5± 2.7 nm/mV). The different phases on the RL and TM over the IHCs and OHCs result in a volume change in the subtectorial space above the IHCs. With a simple hydrodynamical model, radial fluid motion over the inner sulcus cells, IHC and pillar cells, synchronised with the elongation of OHCs, was determined. These results have important implications for the mode of stimulation of IHC stereocilia.

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329 Phase Locking in Mouse Inner Hair Cells: A Model Study

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Transformation of acoustical stimuli in inner hair cells (IHCs)is known to phase lock to the stimulus up to several kHz (Palmer and Russell, 1986, Hear. Res., 24:1-15). This ability is impressive since already in the IHCs the transduction process contains several time-dependent steps from the deflection of the stereocilia to the release of neurotransmitter. The deflection of the stereocilia opens transduction channels which let K^+ enter. The resulting depolarizations activates voltage gated Ca^{2+}-channels. The entering Ca^{2+} ions trigger the release of neurotransmitter, which then leads to post-synaptic currents in the afferent nerve fibers. Repolarization of the IHCs is mediated by several different types of K^+ currents.

To investigate the influence of the composition of the different ionic currents on the ability to phase lock to the stimuli we refined a computer model for mouse IHCs by including a model for the transduction current based on Géléoc et al. (1997, Proc. Roy. Soc. Lond. B, 264:611-621). The results suggest that the large conductance Ca^{2+} dependent BK-type K^+ currents are the most important currents for maintaining phase-locking for frequencies of more than one kHz in mature IHCs. Immature IHCs show only poor phase locking due to the lack of the BK-current, as was suggested by Kros et al. (1998, Nature, 394:281-284).

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Signal analysis of neurophonic responses in the owl's nucleus laminaris 330

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Clicks are powerful stimuli for studying auditory processing, because they allow for a characterization of the transfer properties of a system. Nucleus laminaris in the barn owl contains a network composed of axonal delay lines and coincidence-detection neurons that underlies the extraction of an important sound-localization parameter, the interaural time difference (ITD).

To study the properties of the neural network in the nucleus laminaris, we recorded the neurophonic potential in 7 anesthetized barn owls. Clicks, noises and tones were used as stimuli. Frequency tuning curves, ITD tuning curves and click response curves were determined. Clicks varied in duration (20-80 microseconds), level (0 to 60 dB attenuation), and polarity (rarefaction or condensation). The median of the responses to typically 128 click repetitions was analyzed.

The click-evoked neurophonic responses in nucleus laminaris exhibited oscillations will several frequency components, usually one or two prominent low-frequency (< 2 kHz) components and a high-frequency (>2 kHz) component. Only the high frequency component was further analyzed, because it was close to the frequency tuning measured with tones. The responses were be fitted well by either a Gabor or a Gammatone function. Each of these functions was characterized by 6 parameters. It was tested how the three stimulus parameters of level, duration and polarity influence the 6 fit parameters amplitude, width, frequency, offset, latency and phase. A decrease in click level led to a decrease in amplitude, and increases in latency and frequency. Click duration had little effect on the response. Rarefaction clicks had shorter latencies than condensation clicks. These data provide an important precondition for the understanding of the laminaris network in sound localization.

Motion sensitivity in the barn owl’s auditory midbrain 331

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Barn owls are able to catch prey in total darkness due to their remarkable auditory system. From behavioural experiments it is known that they not only localize sound sources, but also perceive the motion direction of them. About 30% of the neurons in the auditory midbrain were found to be motion sensitive when tested with apparent motion stimuli (Wagner & Takahashi 1990). In that study only horizontal moving stimuli where used. We were interested to show that apparent motion stimuli didn’t cause an artefact and wanted to see whether there is motion sensitivity in non-horizontal directions too.

We used individually measured head related transfer functions to construct virtual moving auditory stimuli. The stimuli gave the impression of a smoothly moving white noise. Stationary stimuli were also created. The stimuli were presented via ear phones to the anesthetized animals. We measured the responses of single and multi units. The virtual moving stimuli were applied, centered on the receptive field measured in advance with stationary stimuli.

When tested with smooth moving virtual stimuli some neurons exhibited motion direction sensitivity. We found units with an oblique highest directionallity as well. Both results support the view that these neurons are the basis for the motion sensitivity found in behaviour.

332 Modulation Tuning in the Auditory Midbrain of Gerbils: Band Passes are formed by Inhibition

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In order to understand the nature of periodicity coding we investigated rate codes of neurons in the IC. All investigated neurons had typical tuning curves in response to pure tones and well defined characteristic frequencies. Stimulated with periodically sinusoidal amplitude modulated pure tones they also showed typical tuning to a best modulation frequency (BMF). The response to periodic stimuli was investigated for different time intervals after stimulus onset.

Forty five percent of the BMF-tuned neurons (n=70) showed also responses to multiples of their BMF in time intervals shortly after the onset of the stimuli. Multiples were only observed in neurons tuned to BMF's between approx. 60 and 300 Hz, below and above band pass tuning is also present at stimulus onset.

Models employing coincidence analysis (Langner 1997, 2002) are apporiate to explain this responses to multiples of BMF. The autocorrelation

$$AKF(\Delta\tau) = \int_T dt\ f(t) * f(t - \Delta\tau)$$

has high values if the periodicity of the signal *f(t)* fits to the correlational delay $\Delta\tau$ and also if the delay is a multiple of the signal periodicity. We were able to simulate comb-like modulation transfer functions using this model.

The suppression of reactions to multiples of BMF seem to require a second filter mechanism in addition to coincidence detection. A possible mechanism is an inhibition triggered by the envelope of the signal (Casseday 2000). Such synchronized inhibition could suppress reactions to periodic acoustic signals if the period is twice or more the neurons BMF. The duration of the inhibition τ_I must fulfill the relation: $\tau_{BMF/2} < \tau_I < \tau_{BMF}$. If the BMF-period is larger than ~16 ms the inhibitory input seems to be already present at stimulus onset. Harmonics of high BMFs elicit weak responses because of reduced synchronicity. This explains the restriction to the specified BMF-range.

Further evidence for synchronized inhibition of appropriate duration was found in depressed spontaneous activity following the on-response in units with low BMFs.

A possible source of this inhibition is the ventral nucleus of the lateral lemniscus. This nucleus receives are precisely timed inputs form octopus cells in the cochlear nucleus (Adams 1997) and provides inhibitory inputs to the IC.

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Projections from inferior colliculus to the lateral lemniscus studied in a slice preparation with anterograde tracers 333

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The auditory midbrain nucleus inferior colliculus (IC) is an important integration site for information carried by ascending as well as descending auditory pathways. A prominent feature of its central nucleus (ICC) is the tonotopic organization with lower frequencies represented in dorsal and higher frequencies represented in ventral ICC. Evidence for a topographic representation of periodicity information was derived from electrophysiological studies (Langner et al., Hearing Res. 2002). Neurons responding best to low modulation frequencies were located medially, whereas those responding best to high modulation frequencies were located laterally. IC receives input from the tonotopically organised nuclei of the lateral lemniscus (LL) and also projects back to them (Malmierca et al., J.Neurosci. 1998). The ventral nucleus of the lateral lemniscus (VNLL) is involved in processing monaural information whereas the dorsal nucleus (DNLL) belongs to the binaural system. We were interested in the spatial pattern of projections between ICC and LL. As it was intended to inject different tracers into nearby locations, this tracing study was performed in brain slices in which injections could be controlled visually.

Crystals of fluororuby and/or fluoroemerald were inserted into ICC in 3000 µm thick transversal slices gained from young adult gerbils. In cases of double injections, tracers were placed into nearby loci covering different areas of the tonotopic respectively periodotopic map. After injections, slices were transferred to a submerged chamber with cold ACSF where they remained for 18 to 24 hours. After fixation in 4% PA, slices were sectioned to a final thickness of 50µm on a cryostat.

Fluororuby and fluoroemerald were transported exclusively in the anterograde direction in this midbrain preparation. In ICC projections were preferentially directed along frequency band laminae and to a lesser extent orthogonal to them. In rostral ICC, fibers leaving towards the commissure, LL and brachium were most prominent. Most of the descending fibers which proceeded to LL left ICC laterally. Thick axons were running

through DNLL and VNLL in the dorsoventral direction, while fewer axons were thin and had regular swellings, likely representing en-passent synapses. From some of the thinner axons collaterals that showed characteristics of terminal arborizations were deviating in the horizontal direction. The latter were only found if larger tracer crystals were used. In VNLL horizontally deviating fibers and terminal arborizations were located in the dorsal two thirds of the nucleus. Axons originating in dorsal parts of ICC (low frequeny region) projected to lateral VNLL, whereas those originating in ventral ICC (high frequency region) projected to medial areas of VNLL. When comparing projection patterns from injection sites differing in mediolateral position in ICC no consistent differences were observed in VNLL. The present findings open new possibilities for further investigation of the as yet poorly understood spatial tonotopic and periodotopic organization of VNLL by studying the fine structure of its reciprocal connections with ICC.

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334 Experimental Tinnitus Induction and Acoustic Stimulation Led to Distinct Patterns of Arg3. 1/Arc and C-fos Expression in the Auditory and Limbic System of the Gerbil

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Subjective tinnitus is a phantom auditory percept often associated with hearing deficits. It can also be induced by high doses of salicylate. Systemic salicylate application reduces activity in auditory brainstem, but increases activity in auditory cortex and amygdala of mongolian gerbils (Wallhäusser-Franke & Langner, Neuroforum 2001). We investigated if neuronal plasticity might be involved in tinnitus formation examining the expression of arg3.1 (also called arc, activity regulated cytoskeleton associated protein). Expression of arg3.1 is driven by an NMDA-receptor dependent mechanism (Link et al, PNAS 1995) and is supposed to be involved in morphological changes of the postsynaptic membrane in response to excitation. C-fos immunohistochemistry was performed in the same preparations.

To induce tinnitus, one group of animals (n=6) received a systemic injection of sodium salicylate (350 mg/kg bodyweight) followed by perfusion 5h later. The second group was exposed for 10 min to narrow band white noise centered at 8kHz (n=3) or 1kHz (n=3) with an intensity of 80 dB SPL and perfused 3h after stimulation.

Arg3.1 immunoreactivity was confined to the forebrain. Noise exposure evoked Arg3.1 expression bilaterally in primary auditory fields AI and AAF, and was focussed to areas representing the frequency content of the stimulus. After salicylate injections labeled neurons were also found bilaterally. They were predominantly organized in clusters extending along isofrequency contours. Salicylate injections led to arg3.1 expression in numerous neurons of central (CeA) and lateral nuclei of the amygdala. In contrast after acoustic stimulation, numbers of labeled neurons were negligible in CeA and less frequent in the lateral parts of the amygdala.

In the same brains, c-fos-immunoreactive neurons were observed in ventral cochlear nucleus only after stimulation not after salicylate injections. In AI and AAF, fos-expressing neurons were far more abundant than arg3.1-expressing ones and were distributed over all frequencies and all cortical layers. In the amygdala the total amount of fos- immunreactive cells again outnumbered that of arg3.1 after salicylate injections, whereas numbers of fos- and arg3.1-expressing neurons did not differ as much after acoustic stimulation.

In accordance with previous findings, these results indicate a reduction of input from the cochlea after the tinnitus-eliciting salicylate injections, and at the same time activation of auditory cortex. Moreover, fos-expressing neurons are always more numerous suggesting that many neurons are activated while only few undergo dendritic morphological changes. We interpret these results in favour of our model of central tinnitus generation which hypothesizes that hyperactivity in auditory cortex arises through positive feedback with the amygdala which should augment during aversive situations and stress (Wallhäusser-Franke and Langner, 2001; Langner and Wallhäusser-Franke Proc.6th Int. Tinnitusseminar, 1999). *Supported by the State of Hessen and a prize of the Messer Foundation*

Periodotopic organization of the ventral nucleus of the lateral lemniscus in the gerbil. 335

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Periodic envelope modulation is an important feature of many acoustic signals. In humans such signals may elicit the percept of pitch. In central inferior colliculus (ICC), a major nucleus for auditory integration, frequency and periodicity information converge on a tonotopically organized network. Electrophysiological investigations showed that temporal information about the envelope of complex tones as present in the auditory periphery is degraded in the ICC and transformed into a rate-place code. Accordingly, units in the ICC were found to be tuned to different modulation frequencies and to be arranged in periodicity maps arranged orthogonal to the tonotopic map.

As a basis of this organization, we suggest a neuronal correlation analysis based on coincidence of delayed and undelayed responses to the modulations in the inferior colliculus (ICC; Langner et al., Hearing Res. 168, 2002). In line with a correlation analysis we observed comb-filters in neurons of the ICC during the first 30 ms of their responses to periodic signals. However, after this initial phase the filter curves were transformed into band-pass filters. We assume that the source of the underlying inhibition is the ventral nucleus of lateral lemniscus (VNLL). As our tracer studies revealed that VNLL receives input from the ICC, we investigated the VNLL also for a systematic representation of periodicity information.

For that purpose we used the 2-deoxyglucose technique. Stimuli were harmonic complexes with fundamental frequencies ranging from 40 to 800 Hz and cut-off frequencies at the low (0.4 - 5 kHz) and the high frequency border (2 - 8 kHz) of their broad band spectra. To avoid distortions an electrostatic speaker (STAX) and low intensities (0 - 55

dB SPL) were used. Animals were placed singly in a soundproofed chamber and were stimulated for 1h.

Our results show that the VNLL is distinctly labelled by different harmonic sounds. This is in line with the assumption of activation by the demonstrated descending projections from the ICC. Moreover, along the longer axis of the VNLL low periodicities are represented dorsally and high periodicities ventrally with a gradient of about 0.28 mm/octave. Taken together with the analysis of the fine structure of the glucose labels this suggests a helical periodicity map in the auditory system of the gerbil reminding of the well-known pitch helix of music psychologists. The results are also in line with the finding of a helical organization of the VNLL of rat (Merchán and Berbel, J. Comp.Neurol. 372, 1996).

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336 Prewired for echolocation? - Auditory cortex responses in young mustached bats

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The auditory cortex of the adult mustached bat is well studied and a model system for cortical processing of echolocation signals. To capture wing-beating prey insects in densely cluttered environment, the bat uses CF-FM echolocation calls with the second harmonic constant frequency component (CF2) at about 61 kHz and is able to resolve small Dopplershifts in returning echoes. In the auditory cortex there are several areas responsive to combinations of echolocation signal components. Sharply tuned neurons whose responses are enhanced by combinations of the first harmonic CF-component of the emitted call and higher harmonic CF-components of the echo are found in the CF/CF region (Suga et al., 1978, Science 200:778-781). Subadult mustached bats of an age between 6-26 days were captured in a hot cave in Cuba. The bats normally require a time span of about 5 weeks to be able to leave the cave and to hunt insects. During this time the CF2 frequency of the echolocation calls and the frequency of a cochlear resonator involved in sharp tuning shift from about 48 to 61 kHz. We mapped the primary auditory cortex of young bats. There were sharply tuned responses to the individual CF2-frequency. Even in the youngest bat which did not echolocate in captivity, neuronal Q10dB values of close to 100 could be found at a neuronal best frequency of 52.6 kHz, which may correspond to the cochlear resonance frequency at this developmental stage. In addition, in the older animals some neurons were already CF/CF combination sensitive. None of the animals tested was yet capable of active flight. This raises the question to which degree cortical circuitry specifically important for echolocation can develop intrinsically without the animal being involved in flight and prey capture, and how such properties depend on the maturation of peripheral stages of the auditory system. Supported by the Volkswagenstiftung, the DFG, and the Royal Society.

A possible vocal-audio interface in the squirrel monkey's brainstem 337

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Squirrel monkeys (*Saimiri sciureus*) often respond to vocalizations of conspecifics with vocalizations of the same type with short latency (100-200 ms). As they never echo their own self-produced vocalizations, this means that there must be a mechanism which allows the animal to distinguish self-produced from external vocalizations within very short time. A possible way to master this problem would be to send a corollary discharge of the vocal motor command to the auditory system, informing the latter about a forthcoming acoustic event.

We recorded the neuronal activity of the peripheral and central nuclei of the inferior colliculus (IC) and of the bordering tegmental and pontine reticular formation (FRTM, FRPc) during vocalization and acoustical stimulation, in order to find out wether these structures are part of a vocal-audio feedforward mechanism.

Vocalization was elicited by injection of kainic acid into the periaqueductal gray (PAG) of the midbrain. The utterances were indistinguishable from spontaneously produced vocalizations.

Our results show that neurons increasing their activity to external acoustic stimuli as well as in advance to self-produced vocalizations are only found in the external and dorsal nucleus of the IC and the adjacent FRTM, but not in the central nucleus of the IC. This points to a possible vocal-audio interface function of the peripheral IC.

The experiments were approved by the animal ethics committee of the district government of Braunschweig, Lower Saxony.

Efferent projections of the ventral paralemniscal area in squirrel monkeys *(Saimiri sciureus)* 338

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It is generally accepted that the periaqueductal grey (PAG) of the midbrain plays an important role in vocal motor control. An earlier study has shown that vocalization electrically elicitable from the PAG can be blocked by injections of the glutamate antagonist kynurenic acid into the ventrolateral pons. This made clear that the descending vocalization pathway does not run directly from the PAG to the phonatory motoneurones, but has at least one additional relay station in the ventrolateral pons. The present study serves to clarify the anterograde projections of this pontine vocalization-blocking site. For this purpose, the anterograde tracer biotin dextranamine was injected into ventrolateral pontine sites capable of blocking PAG-induced vocalization in four squir-

rel monkeys. In one animal, in addition, the retrograde tracer horseradish peroxidase was injected into the cricothyroid muscle for better identification of the nucleus ambiguus, that is, the site of the laryngeal motoneurones. It turned out that none of the animals showed a direct projection to the nucleus ambiguus. There were, however, terminals in the facial nucleus bilaterally and, sparsely, in the trigeminal and hypoglossal nuclei ipsilaterally. Clear terminal labeling was found in the reticular formation surrounding the nucleus ambiguus, a region that has been shown in other studies to be directly connected with the laryngeal motoneurones. Projections also were found into different stations of the auditory pathway, such as the cochlear nuclear complex, superior olive, nucleus trapezoidalis, ventral and dorsal nuclei of the lateral lemniscus, inferior colliculus and medial geniculate body. Additional structures receiving direct input were the peripeduncular, mediodorsal and ventralis posteromedial nuclei of the diencephalon, the reticular formation, PAG and superior colliculus of the mesencephalon, the main sensory trigeminal nucleus, lateral parabrachial nucleus, nucl. reticularis tegmenti pontis, pontine grey and reticular formation in the pons and the spinal trigeminal nucleus, solitary tract nucleus, vestibular nuclear complex, nucl. gracilis, nucl. cuneatus, nucleus reticularis lateralis and ventral raphe in the medulla. These findings make clear that the pontine relay station of the vocalization pathway is only indirectly connected with the laryngeal motoneurones. Which of the many projection areas are in fact involved in vocal control needs additional investigations, using the single-unit recording technique.

339 Neuronal activity in the external nucleus of the inferior colliculus and bordering tegmentum telemetrically recorded during vocal communication in squirrel monkeys *(Saimiri sciureus)*

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Squirrel monkeys, like numerous other species, often respond to vocalizations of conspecifics with vocalization of the same type with very short latency. As the animals never echo their own self-produced vocalizations, it must be assumed that there is a mechanism which allows the animal to distinguish self-produced from external vocalizations within very short time. One possible way to master this problem is to send a corollary discharge of the vocal motor command to the auditory system, informing the latter about the forthcoming acoustic event. The present study is an attempt to find out whether the pericollicular region contains neurones which react differently to self-produced and external vocalizations. Neuronal activity was registered in freely moving squirrel monkeys during spontaneous vocal communication, using a recently developed telemetric single-unit recording technique. Our results show that neurones in the central nucleus of the inferior colliculus do not react differently to self-produced and group mate-produced vocalization of the same type. In the external nucleus of the inferior colliculus and the immediately bordering tegmentum, however, in addition to classical auditory neurones, neurones were found which were activated by external vocalizations and inhibited by self-produced vocalizations. Furthermore, in the paralemniscal zone bordering the dorsal nucleus of the lateral lemniscus ventrally, neurones were found which fired during self-produced vocalization, but not during external vocalization.

These cells became active before vocalization onset and showed a discharge pattern which reflected the amplitude/frequency modulation of the produced vocalization. These findings suggest that the pericentral colliculus inferior and bordering tegmentum receive an input from vocalization-controlling structures which allows them to differentiate self-produced from external vocalization.

Vocalization-related afferents to the midbrain periaqueductal grey in squirrel monkeys (*Saimiri sciureus*) 340

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The midbrain periaqueductal grey (PAG) is assumed to play an essential role in the vocal expression of emotional states. In the present study, we investigated the afferent connections of vocalization-eliciting sites of the PAG in the squirrel monkey (*Saimiri sciureus*). Vocalization was elicited by injection of the glutamate agonist homocysteic acid into the PAG. Tracing of the afferent connections was carried out by injecting wheat germ agglutinin- conjugated horseradish peroxidase (WGA-HRP) into the vocalization-eliciting sites. In order to find out, whether sites producing different call types receive their input from different brain areas, we compared the retrogradely labeled areas in three groups of two animals each. In one group, WGA-HRP was injected into sites yielding "shriek" calls. Shrieking represents a defensive threat call; it is accompanied by a highly aversive emotional state, when tested in a self-stimulation situation. The second group was injected into sites yielding "cackling" calls. Cackling normally occurs during social mobbing; it expresses a moderately aversive emotional state. The third group was injected into sites producing "chuck" calls. Chuck represents a non-aversive close-distance contact call. The results show that there is a virtually complete overlap in the retrogradely labeled structures in the three groups, with the heaviest labeling found in the dorsomedial prefrontal cortex, anterior cingulate cortex, nucleus of the anterior commissure, preoptic region, medial hypothalamus, zona incerta, substantia nigra, locus coeruleus, paralemniscal zone, large parts of the reticular formation and the solitary tract nucleus. The number of retrogradely labeled cells differ greatly between the groups, however. While some structures show the highest relative number of labeled cells in the chuck-producing animals (e.g. nucleus accumbens, anterior septum), others show the heaviest labeling in shrieking animals (e.g. dorsolateral prefrontal cortex, dorsolateral midbrain tegmentum) or cackling animals (e.g. subcallosal gyrus, supramamillary hypothalamus). Some structures show a heavier labeling in two groups, in comparison to the third (for instance chuck and cackle against shriek in the anterior cingulate cortex or cackle and shriek against chuck in the pregeniculate grey). Still others show equal labeling in all three groups (e.g. central amygdala and dorsomedial hypothalamus). These findings make clear, that PAG sites controlling different call types receive partially segregated inputs.

341 Telemetric recording of vocalization-correlated single-unit activity in the ventrolateral pontine brainstem of freely-moving squirrel monkeys (*Saimiri sciureus*)

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Vocalization is a complex behavior pattern consisting of laryngeal, respiratory, and articulatory activity. While there is good knowledge about the muscles involved in phonation and the location of the laryngeal, respiratory, and articulatory motoneurons, very little is known about the way these motoneurons are coordinated in their activity to accomplish a specific vocal pattern. In an earlier study, it has been found that injection of kynurenic acid, a glutamate antagonist, into the ventrolateral pontine brainstem (VLPB) blocks vocalization electrically elicitable from the periaqueductal grey (PAG; Jürgens, 2000). This means that the VLPB forms part of the descending periaqueductal vocalization pathway.

Here we use a miniature telemetric system, which allows simultaneous measurements of single-unit activity and spontaneous uttered vocalization in freely moving monkeys within their social group (Grohrock et al., 1997). We ask, whether there exist additional regions, beside the pools of motoneurons, in the VLPB, particularly the area around the ventral nucleus of the lateral lemniscus and the periolivary region, which are involved in vocal motor control and, if occurring, how neuron response properties are correlated with phonation.

First results show, that neurons in the VLPB respond in correlation with spontaneously uttered vocalizations of the experimental animal. So far, the following types of neural activity could be recorded. The neural activity in one type of neuron was inhibited during auditory stimuli and showed an off-response. During self-produced vocalization, the same kind of activity was seen, however, with inhibition starting before vocalization onset. Consequently a connection between the auditory and vocalization pathway must exist at the pontine level. Other types of neurons responded to self-produced vocalization, but not to auditory stimuli. So, neurons could be found, showing neural activity before and during vocalization or only before vocalization onset. Furthermore, neurons could be recorded increasing their activity before and during vocalization as well as during mastication. Finally, neurons were found, firing during a single call type only, with no activity during all other calls uttered. One type fired exclusively before and during “kecker” calls, another before and during “trill” calls. This suggests that the VLPB plays an important role in the descending periaqueductal vocalization pathway.

Grohrock P, Häusler U, Jürgens U (1997) J Neurosci Methods 76, 7-13
Jürgens U (2000) J Acoust Soc Am 108, 1393-1396
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Topographic representation of frequency-sweep direction in the inferior colliculus of the mouse (*Mus domesticus*) 342

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Frequency sweeps are prevalent in communication calls of bats and often the only component of their echolocation calls. Also, in communication calls of mammals including humans, frequency modulated tones (FM-components) play an important role for call or speech-sound discrimination and recognition. Since mice have both upward and downward FM-components (FM-sweeps) in their communication calls (Ehret, 1989), we assume that there should exist an appropriate neuronal mechanism for identification of sweep direction in the auditory system.

Here we used electrophysiological single-unit mapping to study neuron responses to upward and downward sweeps (at three different sweep velocities: 300 kHz/s, 600 kHz/s, 3 MHz/s) as a function of neuron position in the central nucleus of the inferior colliculus (ICC) of the mouse (outbred strain NMRI). We ask whether preferences for sweep direction follow regular spatial gradients (maps) on isofrequency planes of about 20 kHz in the ICC (Stiebler and Ehret, 1985) and, if maps occur, whether they are compatible with the spatial distribution of sweep direction in the primary auditory cortex (Heil et al., 1992).

The results from 67 neurons showed, that 56.7% of them did not demonstrate response preferences (as estimated by an average rate criterium) to sweep direction at all. Downward sweeps were preferred by 25.4% of the neurons and upward sweeps by 17.9%. Not a single neuron preferred both sweep directions at any of the sweep velocities. We found a spatial separation of neurons preferring upward or downward frequency sweeps on the 20 kHz isofrequency plane of the left ICC. Neurons preferring upward sweeps are located medially and laterally, neurons preferring downward sweeps are located centrally. Neurons with no preference to sweep direction were evenly distributed on the isofrequency plane. This spatial clustering of sweep-direction patterns in the ICC closely mimics FM-direction preferences of neurons on isofrequency strips of the primary auditory cortex.

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Heil P, Rajan R, Irvine DRF (1992) Hear Res 63, 135-156
Stiebler I, Ehret G (1985) J Comp Neurol 238, 65-76
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343 The effect of periaqueductal grey blockade on vocalization elicited from the lower brainstem in the squirrel monkey (*Saimiri sciureus*)

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The periaqueductal grey of the midbrain (PAG) is assumed to play an important role in vocal control. Its electrical and pharmacological stimulation produces vocalization. Its destruction causes mutism. Single-unit recording in the PAG reveals vocalization-correlated activity. In order to find out whether the PAG is the site of vocal pattern generation or rather serves the gating of vocal reactions, we have tested the effects of PAG inactivation on the electrical elicitability of vocalization from different brain regions. In six squirrel monkeys (*Saimiri sciureus*), altogether 64 vocalization-eliciting electrode positions in forebrain, midbrain, pons and medulla were tested before and after injection of muscimol (0.4 µg/µl), a GABA agonist, into the PAG. It turned out that only vocalization sites in the forebrain (anterior cingulate cortex, perifornical hypothalamus) and rostralmost mesencephalic reticular formation could be blocked by PAG inactivation, while vocalization sites in the caudal midbrain, pons and medulla were not affected. It is concluded that the PAG is not the site of vocal pattern generation, as species-specific calls can still be elicited by electrical stimulation of the lower brainstem after inactivation of the PAG.

344 Subcortical projections of the motorcortical larynx area in the rhesus monkey *(Macaca mulatta)*

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In order to better understand the descending voluntary vocal control pathway, we have studied the efferent subcortical projections of the laryngeal motorcortex in the rhesus monkey *(Macaca mulatta)*. For this purpose, the left motorcortex was explored in 3 animals under narcosis. By electrical brain stimulation, sites were identified yielding vocal fold adduction. Effective sites were injected with the anterograde tracer biotin dextranamine. After a survival period of 7 weeks, the animals were sacrificed and the brains processed histologically. Subcortical projections could be traced within the forebrain to the putamen, caudate nucleus, claustrum, zona incerta, field H of Forel and a number of thalamic nuclei, with the heaviest projections to the nuclei ventralis lateralis, ventralis posteromedialis, including its parvocellular part, medialis dorsalis, centralis medialis, centrum medianum and reuniens. In the midbrain, labelling was found in the deep mesencephalic nucleus. In the lower brainstem, fibers terminated in the pontine and medullary reticular formation, locus coeruleus, nucleus subcoeruleus, medial parabrachial nucleus, nucleus of the spinal trigeminal tract, solitary tract nucleus and facial nucleus. No projections were found to the nucl. ambiguus. The fact that monkeys, in contrast to humans, lack a direct connection of the motorcortex with the laryngeal moto-

neurons suggests that this connection has evolved in the last few million years, and might represent one of the factors that made speech evolution possible.

The effect of periaqueductal grey blockade on vocalization elicited from the lower brainstem in the squirrel monkey 345

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The periaqueductal grey of the midbrain (PAG) is assumed to play an important role in vocal control. Its electrical and pharmacological stimulation produces vocalization. Its destruction causes mutism. Single-unit recording in the PAG reveals vocalization-correlated activity. In order to find out whether the PAG is the site of vocal pattern generation or rather serves the gating of vocal reactions, we have tested the effects of PAG inactivation on the electrical elicitability of vocalization from different brain regions. In six squirrel monkeys (Saimiri sciureus), altogether 64 vocalization-eliciting electrode positions in forebrain, midbrain, pons and medulla were tested before and after injection of muscimol (0.4 μg/μl), a GABA agonist, into the PAG. It turned out that only vocalization sites in the forebrain (anterior cingulate cortex, perifornical hypothalamus) and rostralmost mesencephalic reticular formation could be blocked by PAG inactivation, while vocalization sites in the caudal midbrain, pons and medulla were not affected. It is concluded that the PAG is not the site of vocal pattern generation, as species-specific calls can still be elicited by electrical stimulation of the lower brainstem after inactivation of the PAG.

Establishment of a catalogue of expressed genes in the rat auditory brainstem by SAGE 346

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The superior olivary complex (SOC) is an important processing center in the auditory brainstem. It is the first binaural structure in the auditory pathway, i.e., it receives input from both cochleae. The SOC consists of several nuclei, such as the lateral superior olive, the medial superior olive, and the medial nucleus of the trapezoid body. These nuclei are involved in sound localization by computing time or level differences between the two ears. In the past, a large body of electrophysiological studies has been performed in order to unravel the neuronal mechanisms of binaural sound localization. However, the genes which give rise to the molecular elements of this machinery and which ultimately determine these processes are largely unknown. The aim of this project is to establish a catalogue of expressed genes in the SOC employing the technique of serial analysis of gene expression (SAGE). This technique is based on the position-specific generation of a unique 14 bp sequence tag from each transcript. These tags are ligated to concatemers of 300-800 bp in length prior to cloning and sequence analysis. Therefore, each sequencing reaction yields information about >40 expressed genes on

average. We dissected 9 SOCs of 60-day-old rats from brainstem slices under visual control and subjected them to the SAGE protocol. A crucial, yet so far inefficient, step during this procedure is the cloning of the concatenated tags. To improve the efficiency, we first replaced the commonly used cohesive-end by a blunt-end cloning procedure. This modification has proven to be very efficient and robust, and resulted in the generation of more than 500,000 clones. So far, 379 tags have been sequenced and analyzed. 299 tags corresponded to nucleus-encoded and 80 tags to mitochondria-encoded genes. The analysis of the nucleus-encoded transcripts led to the identification of 25 genes and more than 100 expressed sequence tags. One third of the tags displayed no annotation at all. The 25 genes encode proteins from various subcellular compartments, such as the cytoplasm or the plasma membrane. The most abundant nucleus-encoded gene detected so far is the myelin basic protein (7 counts), followed by α-tubulin, SNAP-25 and the Na^+/K^+-dependent ATPase β-1 (3 counts each).Taken together, these data indicate that the SAGE approach will allow us to unravel proteins important for synaptic transmission and intracellular signaling. Thus, the generated SAGE library may be a key to identify the proteins that underlie the neuronal mechanisms of binaural sound localization.

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347 Protein identification in the rat auditory brainstem by 2D-gel electrophoresis and mass spectrometry

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The mammalian auditory brainstem consists of a complex network of nuclei. Many of these nuclei process specific qualities of the information provided by a sound stimulus. For example, the lateral superior olive and the medial superior olive, two major nuclei of the medullary superior olivary complex (SOC), encode interaural differences in sound level and time delay, respectively. This information is integrated in the inferior colliculus (IC), a higher order nucleus situated in the midbrain, and other "higher" structures, enabling the localization of a sound source.

Basically, the function of any given cell or tissue is determined by its repertoire of proteins, i.e. the proteome. Therefore, the analysis of the proteome of SOC and IC should reveal candidate proteins underlying the functions of these processing centers. A powerful method for proteome analysis is the combination of two dimensional gel electrophoresis (2D-GE) with mass spectrometry (MS). This allows the display and identification of most protein variants, including posttranslational modifications.

To analyze the proteome of SOC and IC, these tissues were prepared from brainstem slices taken from several P60 rats. After subcellular fractionation, the protein content of various fractions was analyzed by 2D-GE. For analytic 2D-GE, 200 µg of the respective fraction were separated in the first dimension on a 18 cm long pH 3-10 IPG strip. The second dimension consisted of a 20 x 20 cm^2 gel. The separated proteins were silver stained and scored visually. Each gel displayed several hundred distinct spots. Different fractions displayed clearly distinguishable patterns, and about 5% differences were seen

between the corresponding fractions of SOC and IC. To identify proteins by MS, preparative 2D-gels were prepared, using 500 µg protein, and stained with colloidal coomassie blue. Single spots were cut out, subjected to in-gel tryptic digest, and the resulting peptide mixture was introduced into a quadrupole/time of flight tandem mass spectrometer by nanoelectrospray-ionization. This method allows us to obtain amino acid sequence and mass fingerprint information of the protein, which is then used in database searches to identify the protein with high confidence. So far, we have identified 50 spots from the cytosolic fraction of the SOC proteome. The spots map to proteins of the glycolytic pathway and the cytoskeleton, but also to brain-specific proteins (e.g. GFAP and Neurofilament) and members of intracellular signaling pathways (e.g. Rho-GDP dissociation inhibitor, Phosphatidylethanolamine-binding protein). The majority of the proteins were common to both SOC and IC, but some isoforms were shown to be SOC specific by a limited analysis of the corresponding IC fraction. We conclude that our approach is capable of displaying both common and differentially expressed proteins of defined brain areas and should therefore be helpful to reveal the molecular machinery underlying their functions.

Regulation of intracellular chloride concentration in neonatal lateral superior olive neurons of the mouse. 348

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In lateral superior olive (LSO) neurons of the rat auditory brainstem, glycine receptor-mediated responses change from depolarizing to hyperpolarizing during the first two postnatal weeks (Kandler and Friauf 1995, J. Neurosci. 15:6890-6904). The depolarizing effect of glycine is due to a high intracellular chloride concentration $[Cl^-]_i$, which induces a reversal potential of glycine (E_{Gly}) more positive than the resting membrane potential (V_{rest}). In older LSO neurons, the hyperpolarizing effect is due to a low $[Cl^-]_i$ (Ehrlich et al. 1999, J. Physiol. 520:121-137),

The Na^+ -driven, loop diuretic-sensitive Cl^- transporter NKCC1 was proposed as a major candidate for the inward-directed Cl^- pump in several systems (Russell 2000, Physiol. Rev. 80:211-76). However, our earlier single-cell RT-PCR experiments showed the lack of NKCC1 mRNA in rat LSO neurons at postnatal day (P) 3 (Balakrishnan et al. 2003, J. Neurosci. in press). This study aimed to elucidate the molecular mechanism behind the regulation of high $[Cl^-]_i$ early in development.

Gramicidin-perforated patch-clamp recordings in LSO neurons of acute mouse brainstem slices showed a similar developmental shift in the E_{Gly} around P8 like in rats. The loop diuretic drug bumetanide (30 µmolar), a blocker of NKCC1, had no effect on E_{Gly} of LSO neurons. However, in hippocampal pyramidal neurons from CA1, bumetanide significantly shifted E_{GABA} towards more negative values, which served as a positive control. Furthermore, in NKCC1 knockout mice, E_{Gly} was not significantly different to that observed in wild type-mice. These results emphasize that NKCC1 is not likely to play a role in setting $[Cl^-]_i$ in LSO neurons.

In order to check the possibilities of other Na^+ -dependent Cl^- accumulations, the effect of extracellular Na^+ ($[Na^+]_o$) on $[Cl^-]_i$ was studied. A reduction of $[Na^+]_o$ of 82% showed no effect on E_{Gly} in LSO neurons. In contrast, in control experiments with hippocampal pyramidal neurons, we found a clear negative shift in E_{GABA} upon reduction of $[Na^+]_o$.

In another set of experiments we tested the role of the brain-specific Cl^-/HCO_3^- exchanger AE3 in setting $[Cl^-]_i$ in LSO neurons. First, 200 µmolar DIDS, a blocker of AE3, significantly shifted E_{Gly} towards more negative values at P3. Second, western blots of neonatal auditory brainstem slices confirmed the expression of AE3.

Our results indicate that neither NKCC1 nor another Na^+ -dependent Cl^- accumulation mechanism is involved in $[Cl^-]_i$ regulation in young LSO neurons, whereas it is likely that AE3 plays a role as an inward Cl^- transporter.

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349 Novel inputs to the superior olivary complex of the rat revealed by optical recordings with voltage-sensitive dyes

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The superior olivary complex (SOC) is the first station in the ascending auditory pathway where information converges from both ears. The principal nuclei of the SOC are the medial nucleus of the trapezoid body, the lateral superior olive (LSO), the medial superior olive (MSO), and the superior paraolivary nucleus (SPN). The bilateral information processing in the SOC is vital for sound localization. Nonetheless, the underlying neuronal circuits have not been completely revealed so far. In order to study spatiotemporal aspects of information processing in the entire SOC, we employed an optical recording method using the fast voltage-sensitive dye RH-795. These measurements allow the simultaneous monitoring of electrical activity in several nuclei of the SOC as well as the detection of regional differences within a nucleus.

In acute brainstem slices of rats aged between postnatal day 3 and 13, ipsilateral or contralateral inputs to the SOC were stimulated electrically by bipolar electrodes. The optical signals were detected by a 464-photodiode array (Neuroplex, RedShirtImaging, USA). The signal recorded by a given photodiode consisted mostly of two components. These two components were identified as pre- and postsynaptic potentials in experiments with Ca^{2+}-free extracellular solutions. Moreover, the optical signals correlated with morphological structures, i.e., the presynaptic components were prominent in neuropil regions whereas the postsynaptic components dominated in soma regions.

Concerning the ipsilateral inputs to the LSO, we pharmacologically identified a strychnine-sensitive, glycinergic input in addition to the known glutamatergic input. Furthermore, besides the known glycinergic input from the contralateral side, we found a CNQX-sensitive, glutamatergic input. This novel contralateral glutamatergic input was dominant in the dorsal part of the medial limb. In both instances, the amplitude of the principal inputs to the LSO was larger than that of the novel inputs.

Regarding the MSO, electrophysiological experiments with strychnine showed that the anatomically described ipsilateral glycinergic input (Grothe 2000, Prog. Neurobiol. 61:581-610) was functional. Finally, we found the anatomically described contralateral glutamatergic input to the SPN (Friauf and Ostwald 1988, Exp. Brain Res. 73:263-284) to be functional by CNQX experiments.

Our results complement the knowledge about the neuronal circuits within the SOC. In addition, they imply that further experiments with voltage-sensitive dyes will allow us to understand these circuits in more detail.

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The Effect of Bicuculline on Temporal Processing in the Auditory Cortex of the Unanaesthetized Mongolian Gerbil

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To study the contribution of intracortical GABAergic inhibition in temporal processing of sounds, single and multi-unit responses to pure tones and sinusoidally amplitude modulated (AM) tones were recorded from the left primary auditory cortex (AI) of unanaesthetized Mongolian Gerbils before, during and after microiontophoretic application of the $GABA_A$-receptor antagonist bicuculline (BIC). We used 3-barrel glass pipettes (total tip diameter 10-18µm), with one barrel containing BIC, and the other two containing NaCl for recording of neuronal activity and current compensation, respectively. Pure tones and AM tones of 200 to 500 ms duration and with 5 ms linear rise and fall times were presented free field at 65 dB SPL and in random order.

Before the application of BIC, responses of most units to AM tones showed phase-locking to the AM envelope at periodicities below 30 Hz, although in some units we observed phase-locking up to 60 Hz. When $GABA_A$-mediated inhibition was blocked by application of BIC (20-40 nA; 10 ± 2 min), we observed an increase in spontaneous activity and in the duration of onset responses to pure tones. Furthermore, the discharge rates to both pure tones and AM tones increased under the BIC condition and a broadening of the frequency response range to pure tones was observed. Interestingly, any phase-locked discharges that were seen in the AM responses before the application of BIC were eliminated during BIC application, whereas the ON-responses were affected in a similar fashion to the ON-responses to pure tones. All effects of BIC were reversible. The initial responses recovered within approximately 20 to 30 minutes.

We conclude from these results that $GABA_A$-mediated inhibition plays a crucial role in the temporal processing of sounds in AI.

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351 Relating spatiotemporal patterns in the ongoing cortical activity to the interpretation of intracortical microstimulation

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In the development of neuroprosthetic devices, electrical microstimulation in the depth of sensory neocortex might provide a promising alternative to peripheral nerve stimulation (Schmidt et al. 1996, Brindley & Lewin 1968). The lack of success in the development of sensory cortex prostheses may be based on a too naive transfer of unidirectional stimulation procedures operative in peripheral neuroprostheses. We have recently shown that cortical activity underlying cognitive aspects of information processing does occur in form of discernible spatial patterns of activity distribution (so-called 'marked states') only at unpredicted points in time in the ongoing activity and lasts for only a few tens of ms (Ohl et al., 2001). These aspects of information processing pertain to the perceptual sorting of stimuli into meaningful categories and will hence be elemental to the correct interpretation of a stimulus delivered by a sensory cortical prosthesis.

In our preliminary work we applied intracortical microstimulation to the auditory cortex of the Mongolian gerbil via a simple unidirectionally operating cortical implant (Scheich & Breindl 2002). In parallel we recorded an epidural multichannel electrocorticogram. Animals were trained in a GO/(NO-GO)-paradigm to discriminate stimulation sites in the low and high frequency region of the tonotopic map of the primary auditory cortex (Deliano et al. 2002). We analyzed patterns of ongoing cortical activity in relation to electrical microstimulation and its behavioral interpretation. With our work we aim at constructing a novel type of *interactive cortex prosthesis* which controls its operation interactively with the recorded brain state to ensure optimized communication between prosthesis and the nervous system.

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352 A unifying basis of physiological and perceptual detection thresholds in hearing

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Thresholds of auditory-nerve (AN) fibers and auditory neurons are commonly specified in terms of sound pressure only, implying that they are independent of time. At the perceptual level, however, the sound pressure required for detection decreases with increasing stimulus duration in every species examined. Such a trading relationship between a sound´s amplitude, or a related parameter, and its duration is consistent with the notion that the auditory system summates or integrates sound over time. The quantity commonly believed to be integrated is sound intensity, i.e. the amount of sound

power transmitted per unit area which, for pure tones, is proportional to the square of the peak pressure or the pressure envelope. Sound power integrated over time yields acoustic energy, and the common interpretation of the perceptual data therefore implies that the auditory system has a threshold that is best specified in terms of the sound´s acoustic energy density, and not its pressure. However, threshold energy increases with stimulus duration and leaky integrators of intensity with time constants of 100s of milliseconds are required to fit the data. Such time constants are unknown in physiology and are also incompatible with the high temporal resolution of the auditory system, creating the 'resolution-integration' paradox.

Here we demonstrate that AN, auditory cortex and perceptual thresholds are all based on integration of the pressure envelope of the sound, rather than on intensity. The functions relating the pressure envelope integration thresholds and time for AN fibers, cortical neurons and perception in the same species (cat), as well as for perception in many different vertebrate species, are remarkably similar. They are well described by a power law which resolves the resolution-integration paradox. We show that the integrator must be located in the first synapse in the auditory pathway, viz. the inner hair cell - AN fiber synapse. We argue that it is a statistical integrator, based upon the Ca-cooperativity of exocytosis, and that the "integration time" is the waiting time for a sufficient number of such events to occur.

Spectral and Virtual Pitch Processing are Lateralized Differently in Human Auditory Cortex

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Pitch is one of the main perceptual characteristics of sound. Complex harmonic sounds elicit a percept with a pitch that corresponds to the periodicity of the sound. This is still the case when a number of lower harmonics are not contained within the signal (= pitch of the missing fundamental, periodicity pitch, virtual pitch). However, the pitch percept may be ambiguous: depending on the subjects attention it may correspond either to the periodicity or the spectral content of the sound. Using fMRI we investigated the differences between spectral and virtual pitch processing in human auditory cortex (AC).

Sequences of three amplitude modulated tones (AM), where the spectral content was changed either in the same or the opposite direction as the periodicity, were presented to a total of 10 subjects. Subjects were asked to focus their attention either on the spectral or virtual pitch and had to report whether the pitch change of the AM sequence was upwards or downwards. Behavioral performance of all subjects was above 90% correct responses for both attentional conditions. Although the AC showed strong activations in both conditions, the spectral pitch condition showed significantly stronger activations in right secondary AC (Gyrus temporalis superior, Area 42, 22) and right Area 40, whereas the virtual pitch condition gave significantly stronger activations in left secondary AC (Area 42) and left Areas 20 and 21 (Gyrus temporalis medius and inferior). Note that the stimulus material was identical in both conditions. Therefore it seems that a top-down

process, which may be reflected in additional activations we observed in several locations of left prefrontal and left limbic association cortex, selectively activates cortical areas responsible for spectral and virtual pitch processing, respectively. These activations are not only located in different hemispheres (spectal pitch: right, virtual pitch: left), but also include different cortical areas.

354 Early and late electrocorticogram patterns in primary auditory cortex of trained animals

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We studied spatiotemporal patterns of activity in auditory cortex during auditory discrimination training in awake animals (*Mongolian gerbil*). Epidural electrocorticograms were measured using 18-channel (3×6) electrode arrays implanted over the right auditory cortex (field AI) in four animals trained to discriminate between a rising and a falling frequency modulated tone (frequency range 2 to 4 kHz, duration 250 ms). Using a previously introduced classification procedure transient patterns of cortical activity suitable to discriminate between the rising and the falling modulation were identified. *Early* (locked to stimulus onset) and *late* (emerging at variable times after the stimulus) patterns could be differentiated. Deletion of increasing numbers of randomly selected electrodes were used to determine a critical density of recording channels required to capture the discriminative power of the early and late patterns. Statistical analysis of the classification revealed a sigmoid dependence of the discriminative power from the number of remaining electrodes with an inflection point at 12 electrodes. Discriminant analysis of the sets of electrodes deleted revealed that in the early patterns discriminative information was focal on regions corresponding to the tonotopic representation of the stimuli whereas in late patterns this information seemed to be distributed non-focally across larger cortical regions (Ohl et al., *Biol. Cybern.*, in press). This analysis supports the previous notion of the coexistence of topographically organized activity states related to the physical stimulus features and non-topographically organized states largely determined by intrinsic factors (Ohl et al., *Nature*, 2001, 412: 733-736).

355 Comparison of the primary and the caudomedial field of monkey's auditory cortex

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The present study concerns the question why there are multiple representations of the cochlea in the auditory cortex. Recent studies show that the response properties of neurons in different auditory fields are similar. Therefore we speculate that various auditory fields differ with respect to other features and analysed the topographical distribution of various response properties in different auditory fields.

We used a 7-electrode array and recorded at many sites the discharge of small clusters of neurons and field potentials from upper layers of the auditory cortex in 4 anesthetised monkeys (Macaca fascicularis). At each site, response properties were measured by presenting pure tone bursts with frequencies from 0.1 to 32.0 kHz. The best frequency (BF) of a site was determined by finding the tone that elicited the maximal amplitude of a field potential site and the tone that elicited the maximal number of discharges at a site.

At most recording sites the BF of the field potential and the BF of the multiunit response were similar. In primary auditory cortex (A1) and caudomedial field (CM) amplitude and latency of responses were in largely overlapping ranges. In A1 the BF was quite orderly distributed with a monotonic gradient that changed from lower values (0.1 kHz) to higher values (24 kHz) in rostrolateral direction. In CM the gradient was inverted and the distribution was less orderly.

These findings suggest that A1 and CM are functionally specialised with respect to the topographic arrangement of response properties rather than by the range of response properties. We therefore reckon that auditory fields differ in the way the response properties of the neurons in a field are integrated.

A neural network model of the guinea pig auditory cortex for detecting a frequency-modulated sound **356**

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Optical imaging studies using a voltage-sensitive dye have demonstrated specific neuronal responses to a frequency-modulated (FM) sound in the guinea pig auditory cortex. The responses consist of two phases: the initial response spreading widely over the auditory cortex, and the subsequent response which is focused spatially (about 400 microns in diameter) and moves across the iso-frequency bands (Horikawa et al., 1998). This spatially focused response oscillates transiently at about 40 Hz, which is higher than that observed during a pure-tone presentation.

We hypothesized that these spatial focusing and rapid oscillatory activity of the subsequent response to an FM sound were produced by the interaction between excitatory and inhibitory populations of neurons within the auditory cortex. To examine this hypothesis, we developed a neural network model of lateral inhibition in the auditory cortex by modifying the Wilson-Cowan model (Wilson and Cowan, 1972; Yamaguchi et al., 2001).

The neural network model consists of a two-dimensional array of 30x30 units. Two excitatory and inhibitory components are located within each 100-microns square field. The time evolution of a membrane potential at each component is given by a nonlinear differential equation characterized by the summation of inputs from other excitatory and inhibitory components. The distance and weight of the connection between components are adjusted to show lateral inhibition where excitatory effects are in inverse proportion to the distance.

In summary, this model could show the spatial focusing and oscillatory activity of the subsequent response to an FM sound found by the optical imaging. This result indicated that lateral inhibition was sufficient to produce spatial focusing of subsequent responses. But this model also showed two main disagreements with the experimental data: stronger inhibition after each initial or subsequent response, and a lower frequency of the oscillatory activity (20 Hz). These disagreements may be caused by a lack of multiple and different excitatory components as reflected in the NMDA and non-NMDA components.

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357 Differential expression of Ih in inferior colliculus neurons

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The inferior colliculus (IC) comprises a heterogeneous population of neurons that analyses various aspects of sounds. It is generally accepted that the distribution of different ion channels in neurons strongly influences how an individual neuron processes incoming information. E.g. the hyperpolarization-activated current (Ih) has been predominantly found in auditory brainstem neurons that are involved in precise temporal processing (1, 2, 3) and is considered to improve the temporal analysis of inputs (4, 5). However, little is known about a differential ion channel distribution among IC neurons and how it might contribute to the processing of incoming information. Therefore, we tested for the presence of Ih in different classes of IC neurons.

Whole-cell patch clamp recordings were obtained under visual guidance from IC neurons of acute brain slices from P17-P19 rats using standard voltage and current-clamp techniques.

Depending on their spike pattern to positive current step injections, neurons were classified into onset (about 20% of the total population), adapting (about 30%) and sustained (about 50%) firing cells. Onset neurons had significantly lower input resistance and a more depolarized resting membrane potential compared to sustained firing cells. Adapting neurons had properties that were in between onset and sustained firing cells. Upon hyperpolarizing current injection onset neurons exhibited a pronounced depolarizing sag that was blocked by addition of the Ih-antagonist ZD7288 (5 μm) to the bath. Little or no Ih was observed in sustained firing neurons. Ih current amplitude was quantified by

hyperpolarizing the neuron to 1 s long step potentials ranging from –70 to –120 mV using the voltage-clamp technique. At all potentials tested, this current was largest in onset neurons, medium in adapting, and smallest in sustained firing neurons. A large proportion of this current could be blocked by ZD7288 suggesting a significantly larger Ih current in onset cells compared to sustained cells. Activation kinetics were measured by fitting a double exponential curve to the obtained currents. The activation time (fast and slow time constant of the exponential curve) was significantly faster for all voltages in onset neurons compared to sustained neurons whereas adapting cells had similar activation kinetics as onset neurons.

We conclude that a specific population of IC neurons, namely the onset cells, show membrane properties similar to those neurons in the auditory brainstem that are involved in temporally precise sound analysis. We propose that this population of IC neurons also processes temporal acoustic information. Whether their inputs originate in one of these "temporal" auditory brainstem neurons remains to be elucidated.

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Acoustic experience is necessary for natural development of sound localization mechanisms **358**

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Sound localization is one of the most important tasks our hearing system has to perform. Interaural time differences (ITD) are a major cue for low frequency sound localization. In mammals, ITDs are processed in the medial superior olive (MSO), an auditory brainstem structure. A neuron in the MSO receives excitatory and inhibitory inputs from both ears. An excitation-dependent coincidence mechanism sets the basis for an ITD tuning curve that is adjusted to the physiologically relevant range by temporally precise inhibition (1). These inhibitory, glycine-mediated projections undergo a structural development shortly after hearing onset. This development depends on experience of spatial acoustic cues and fails to develop in animals that are reared in omnidirectional white noise designed to mask most spatial cues (2). We therefore hypothesize that early in development, when the excitatory projections are fully established, the inhibitory inputs undergo an experience dependent structural development necessary for proper ITD tuning.

We now show that ITD tuning in juvenile Gerbils, shortly after hearing onset, and in animals that have been exposed to white noise during hearing onset from P10 to P25, is not adjusted to the physiological range, as found in control animals that had normal

auditory experience. ITD tuning in adult animals that have been exposed to white noise, however, is normal.

We therefore conclude that experience of spatial acoustic cues during a critical period is essential for normal development of ITD coding. This exemplifies how neuronal processing can adjust to behaviorally relevant cues.

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359 Dynamics of Neural Sequences in Premotor Areas of the Songbird

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The song-control system of songbirds such as the zebra finch comprises the nucleus interface (NIf), the higher vocal center (HVC), and the nucleus of the archistriatum (RA). These premotor areas are arranged in a feed-forward manner; lesioning any of them leads to a loss of singing ability. We have chronically recorded from identified neurons in singing birds and acutely recorded from pairs of identified neurons in sleeping birds. Our results show that songs are encoded by ultrasparse sequences formed by HVC neurons projecting to RA. Each of these neurons bursts once per song motif and so, as a population, RA-projecting neurons explicitly represent the time in the motif. In sleeping birds, we see a similar sparse code of RA-projecting neurons, relevant for the generation song-replay sequences in RA.

Song production in songbirds is a learned behavior, and relies on both sensory feedback and motor practice in the learning phase. We find that the temporally sparse activity in HVC could play an important role in the rapid learning of premotor representations in RA. We demonstrate this principle by simulating the learning process in a feedforward network of nonlinear neurons, and by analyzing the learning behavior of a network of linear neurons. We show that the time required to learn a song is proportional to the number of times an HVC neuron is active during a song motif.

360 Classification of stochastic impulse responses in echolocation

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Echolocating bats reveal extraordinary capabilities to identify three-dimensional objects exclusively through echolocation. In their natural environment, bats are surrounded by very complex objects often comprising hundreds of sound-reflecting surfaces. Kuc (2001) and Muller and Kuc (2000)investigated the acoustical impulse responses (IRs) of

plants that may be used by bats as landmarks for wide-range orientation. They found that IRs of foliage can only be described stochastically. One parameter suitable for the classification of such complex IRs is the degree of envelope fluctuation of the IR, quantified as its crest factor. A more stable measure of the same IR feature is the 4th moment of the IR. The current psychoacoustical experiments were designed to investigate the ability of the fruit-eating bat, *Phyllostomus discolor*, to discriminate and classify complex IRs which differ in their 4th moment. The bats' ultrasonic emissions were recorded, convoluted with a 16.4-ms complex IR and played back with a delay of 18 ms relative to the emission. Using a two-alternative, forced-choice paradigm with food reward, the bats learned to discriminate a specific IR with a high 4th moment (corresponding to a large envelope fluctuation) from a specific IR with a low 4th moment (corresponding to a small envelope fluctuation). In subsequent experiments, it is tested to which extent the bats can classify unknown IRs according to their degree of envelope fluctuation.

Results are discussed with respect to possible neural processing strategies used to evaluate IRs in echolocation.

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Processing of periodicity by chopping units in the ventral cochlear nucleus

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Elektrophysiological recordings in the cat auditory nerve (AN) have shown that an accurate neural correlate of pitch can be obtained by applying an autocorrelation to the neural discharges (Cariani and Delgutte 1996a; Cariani and Delgutte 1996b). However, it is unclear how a mechanism akin to autocorrelation is implemented in the auditory system. Recordings from Sustained-Chopper (CS) units in the guinea-pig ventral cochlear nucleus indicate that CS units may play an important role in the extraction of neural periodicity (Wiegrebe and Winter 2001; Winter et al. 2001). The current study introduces a realistic computer model of CS units and investigates their contribution to periodicity processing. The simulated units reproduce electrophysiological recordings from guinea-pig CS units with high fidelity in terms of both firing rate and temporal synchrony. Simulated responses include stimulation with pure tones, harmonic complexes with different phase relations, amplitude-modulated noise and iterated rippled noise.

Subsequent simulations investigate how a two-dimensional array of CS units with different best frequencies (BFs) and chopping periods contributes to the extraction of periodicity. The results show that changes in temporal synchrony of firing of a population of CS units with different BFs but a specific chopping period are a reliable neural correlate of the strength of a specific pitch. A computer model based on this two-dimensional

array of CS units predicts the pitch and pitch strength of iterated rippled noise, the dominance region of pitch, the dependence of pitch and pitch strength on the phase relation between the harmonics of harmonic complexes and the weak pitch of amplitude-modulated noise. It is concluded that the neural processing of CS unit represents an important first step to convert the temporal periodicity code, as it is preserved in the auditory nerve, into a rate-place representation of pitch as it is likely to exist in the central auditory system.

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362 Spatial echo suppression in echolocation

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Echolocating bats can localise prey with high spatial accuracy. In an anechoic environment, the time delay between a sonar emission and the direction from which the echo is perceived define the position of the reflecting object unambiguously. However, an ensonified object will not only reflect the sound in the direction of the emitting bat but also into other directions depending on the angle of the reflecting surfaces relative to the bat. Under natural conditions, bats often hunt prey insects in the vicinity of foliage or above water surfaces. Thus, it is highly likely that a bat will hear not only the echo coming directly from the prey object, but also echoes that are first reflected by the prey and then by e.g., the water surface. These higher-order echoes thus produce acoustic mirror images of the prey with misleading spatial information. In humans and other mammals, the law of the first wavefront describes the suppression of the spatial information of echoes to enable accurate sound localisation in reverberant environments (Litovsky et al. 1999). Do bats use a similar auditory processing to suppress the misleading information of acoustic mirror images?

The current psychoacoustical experiments were designed to investigate to which extent echolocating bats spontaneously suppress the spatial information from acoustic mirror images.

The experimental animal was the Indian false vampire bat, *Megaderma lyra* and the lesser spear-nosed bat, *Phyllostomus discolor*. In a two-alternative, forced-choice experiment with food reward, the bats were presented with virtual targets generated by playing back a delayed copy (echo) of the bats sonar emissions through one of two ultrasonic loudspeakers. The bats were trained to fly or walk towards that speaker which emitted the echo. When the bats had learned this task, test trials were randomly interspersed

where both speakers emitted an echo but the echo from one speaker had an additional delay between 0 and 12.8 ms relative to the other speaker. In these test trials, the bats were free to choose any speaker to get a food reward, i.e., the spontaneous performance of the bats was assessed. In *M. lyra*, one of the five individuals tested reliably chose to prefer the speaker emitting the first echo when the echo from the other speaker was delayed by another 0.8 to 3.2 ms, i.e., this bat spontaneously showed highly significant suppression of higher-order echoes. The other four individuals, however, did not prefer one of the two echoes spontaneously.

These data indicate that while *M. lyra* may well be able to suppress the spatial information of higher-order echoes, this is not a mandatory auditory processing strategy and may be recruited only when the bat benefits from this suppression.

Data will also be presented for the bat *P. discolor* using the same experimental paradigm.

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An auditory model for echo suppression based upon dynamic recordings in the gerbil's DNLL 363

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Contralaterally excited and ipsilaterally inhibited (EI) cells in the dorsal nucleus of the lateral lemniscus (DNLL) exhibit a specific response feature called persistent inhibition, when stimulated with dynamic sound bursts moving between the two hemispheres. While the first sound will be answered as expected, it conditions the cell response to subsequent signals arriving within the next 10-25 ms. These effects have been so far only recorded in the bat 1,2,3 and have been confirmed for the Mongolian gerbil DNLL, using acoustic stimuli consisting of three tone pulses with changing interaural intensities.

Based on these findings, a MATLAB/SIMULINK simulation model has been developed and tested using synthetic signals and free-field recordings to reproduce and elucidate the persistent inhibition effect. The simulation architecture includes a cochlear and a hair cell–ganglion model with a 16-channel frequency resolution between 100 Hz and 5 kHz. The entire model is based on dynamic Integrate and Fire neurons and static as well as dynamic synapses, but contains no adaptive or learning elements. It is simulated with a time resolution of 10 mu s. The model includes monaural cochlear nucleus (CN) cells and binaural LSO neurons in both hemispheres, projecting their firing pattern to the DNLL and the inferior colliculus (IC). The DNLL neurons in turn, project contralaterally to specific regions within the IC, where EI units are formed de novo by these inhibitory innervations and the excitatory input from lower centers. Here, the inhibitory

projections from the DNLL modulate the direction selectivity of these IC- EI units during the period of persistent inhibition after the first wave front.

Our model proved capable to suppress echoes from various directions under normal and highly reverberant conditions and to reproduce recordings in the gerbil as well as the bat. The principle effect is the removal of inhibitory GABAergic inputs to specific regions of the IC caused by the persistent inhibition of DNLL cells.

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364 Methods for Mouse Psychoacoustics

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Laboratory mice (*Mus musculus*) are especially suited for studies of auditory physiology since they are easy to breed, multiple mutant lines are known, and the mouse genome has been sequenced. Furthermore, mice age relatively fast and can therefore provide practical models for age-related hearing deficits. Results obtained in neurophysiological experiments and in psychoacoustic tests can be directly compared, leading to a better understanding of the mechanisms involved in auditory processing. Establishing efficient methods is very important for successful auditory research. Therefore we set out to compare the suitability of two different psychoacoustic operant procedures, 2-down-1up adaptive tracking and the method of constant stimuli, for mice. Contrary to previous studies, they require no aversive conditioning and use food deprivation rather than water deprivation (e.g., Prosen et al., 2000, J. Neurosci. Meth. 97: 59-67). The setup and procedure were adapted following methods that proved to be suitable in studying hearing in gerbils (Kittel et al., 2002, Hear. Res. 164: 69-76).

The following results were obtained for the detection of tones in six subjects. Both the thresholds (Wilcoxon two sample test, $p=0.002$) and the standard deviations (Wilcoxon two sample test, $p=0.004$) obtained with the constant-stimuli procedure were significantly lower than those obtained with the adaptive tracking procedure. The range of thresholds among the six subjects did not differ as much in the constant-stimuli procedure as in the adaptive-tracking procedure. Thus, a constant-stimuli procedure appears to be better suited for mouse psychoacoustics. Our constant stimuli sessions consisted of 110 trials. Session length, however, could be reduced without loss of accuracy. If we discarded the first 10 trials (warm-up period), and analysed the next 100, 70 or 50 trials only, the results did not differ significantly from those session with 110 trials, neither in the threshold (Wilcoxon two sample test, $p=0.700$, $p=0.589$, $p=0.699$, respectively) nor in the standard deviation (Wilcoxon two sample test, $p=0.981$, $p=0.234$, $p=0.281$, respectively). Thus, shorter sessions could be used to obtain thresholds without significant loss of accuracy.

Auditory grouping and CMR: Psychophysics and physiology 365

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The European starling (*Sturnus vulgaris*) is a bird species that shows perceptual effects of auditory grouping and scene analysis that are very similar to those observed in humans. Common modulation of signal envelopes as well as common onsets and offsets provide cues exploited by the auditory system for grouping. In this study, we present data from experiments on Comodulation masking release (CMR) comparable to those pioneered by Grose & Hall (1993, JASA 93: 2896-2902) with multiple narrow-band noise maskers.

Four European starlings were trained in a Go/NoGo paradigm to report the detection of a 2 kHz tone (400 ms duration) temporally centered in gated noise maskers. These consisted of 25-Hz-wide bands of noise that were presented either synchronously (all seven masker bands 600 ms duration) or asynchronously (on-frequency band [OFB] centered at 2 kHz, starting 100 ms before and ending 100 ms after the flanking bands [FB] of 600 ms duration) with the masker envelopes being either uncorrelated or coherently modulated. Signal detection theory was applied to determine the birds' detection thresholds; threshold criterion was a d' of 1.8. Masking release for coherently modulated maskers versus uncorrelated maskers was on average about 18 dB for signal detection in synchronous maskers at 10 dB/Hz and 28 dB at 50 dB/Hz masker level. Masking release was significantly reduced by about 5 dB for asynchronous maskers of either spectrum level. FB separation had a small effect on masking release.

We recorded neuronal responses in the input area of the auditory forebrain of awake unrestrained starlings using a similar stimulus paradigm as in the behavioural experiments. The tone was always presented at the neurons' characteristic frequency, and it was centered in the on-frequency band (OFB). Three flanking bands each were either presented above and below the OFB within the limits of the excitatory frequency-tuning curve (FTC), or they were presented in the suppressive side-bands of the FTC. Neuronal masked tone thresholds for synchronously presented noise bands were significantly lower in coherently modulated maskers compared to uncorrelated maskers that were presented within the limits of the neurons' excitatory tuning curve indicating CMR. A reduced masking could also be found for maskers with synchronous flanking bands that were presented in the suppression areas of the tuning curve. Asynchronous onset of the OFB considerably reduced the masking release. These data indicate that response patterns reflecting auditory grouping can be observed at the level of forebrain neurons. For all stimulus conditions, a substantial fraction of the neuronal population exhibited a masking release that was similar to the CMR determined psychophysically in the animals.

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366 Neural mechanisms of auditory scene analysis in the European starling (*Sturnus vulgaris*)

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Auditory scene analysis refers to the process by which the auditory system groups sensory input to form coherent perceptual representations of various sound sources. One example of auditory scene analysis is the "streaming effect" demonstrated in human psychoacoustic studies. Human listeners presented with a rapid and repeated 3-tone sequence comprised of two different tones (e.g., ABA-ABA-ABA-) report hearing a "galloping" rhythm of alternating tones at slow tone repetition rates and when the A and B tones are similar in frequency. At higher tone repetition rates, and with larger spectral differences between the A and B tones, the A and B tones are perceptually segregated into separate auditory streams. Under these conditions, listeners report hearing two separate tone sequences with different "isochronous" rhythms corresponding to two sequences of tones (e.g., A-A-A-A-A-A- and -B---B---B-; dashes correspond to perceived silence within a stream). Using this ABA- stimulus paradigm in a psychoacoustics task, MacDougall-Shackleton et al. (1998, *JASA*, 103:3581-3587) demonstrated the streaming effect in the European starling (*Sturnus vulgaris*). Here we report results from a study of the neural basis of the streaming effect in starlings. We recorded multi-unit activity in the auditory forebrain of awake and freely behaving birds using radio telemetry. We presented birds with repeated tone sequences (ABA-ABA-) in which the frequency of the A tone was specified as the recording site's characteristic frequency. Within a stimulus sequence, the frequency of the B tone differed from that of the A tone by 0, 2, 4, 6, 8, 10, or 12 semitones. Tones within a sequence were presented at one of three durations (25, 40, and 100 ms) and four repetition rates (tone period = 100, 200, 400, and 800% of tone duration). Responses to the B tone exhibited marked suppression at frequency differences above 2-4 semitones, which is well below the 9 semitone difference used by MacDougall-Shackleton et al. to elicit the streaming effect in behavioral tests in the same species. Responses to the B tones were additionally suppressed at the fastest tone repetition rate. Our results suggest that low-level (i.e., non-attentive) auditory processes, such as spectral filtering and forward masking, contribute to the segregation of A and B tone sequences into separate auditory streams.

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367 The pitch of an induced Tinnitus sensation

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In order to examine the neuronal origin and generation mechanisms of Tinnitus in man an animal model is needed to allow invasive *in vivo* and histological studies. Using operant conditioning techniques, a behavioral animal model was developed to characterize the auditory sound experience of rats. Animals were trained to rest still during

silence and to perform actively while a continuous white noise tone was played. After an injection of sodium salicylate (e.g. Aspirin®), animals behaved like perceiving sound even in absence of an external sound source. This behavior could be directly explained by the presence of a phantom auditory experience in the rats (Tinnitus). Unspecific side effects of the drug could be excluded[1)]. For better understanding of how and where the salicylate induced Tinnitus is generated and what the cellular mechanisms are, the frequency characteristic of the phantom auditory experience (pitch) should be known. *Purpose:* In the present study, we focused on the frequency specificity of the rats' Tinnitus sensation. Narrowing down the frequency content of the Tinnitus sensation would give a first hint on the origin within the tonotopically organized peripheral auditory structures (cochlea, Nucleus cochlearis, Colliculus inferior). *Methods:* Animals were trained in a standard behavioral cage (Skinner-box) adapted for the purpose of this study. While a constant tone was played (sound), animals could access liquid feeders to draw a sugar water reward. When the constant tone stopped (silence), no rewards were given and an electrical foot shock of 0.1-0.3 mA was randomly given to the rat when trying to draw a reward during silence. Tinnitus was induced by injecting salicylate (350 mg/kg b.w.). Three hours after injection, animals were tested for their activity behavior during sound and silence in the absence of reward and foot shocks. The animals' sound experience was judged by relating the activity during silence to the activity during sound. Training and testing of 9 Wistar rats was conducted successively with 5, 8 and 13 kHz pure tones and a broadband white noise sound. *Results:* The behavioral effect of the salicylate induced Tinnitus was measured as ratio of activity during silence and sound (SA-ratio). For rats treated with saline, the typical SA-ratios were small (close to 0). For animals treated with salicylate we found SA-ratios of 0.28 (white noise sound), 0.41 (5 kHz), 0.04 (8 kHz) and -0.03 (13 kHz). The data for white noise and 5 kHz revealed statistically significant behavioral changes after salicylate administration, the behavioral data for 8 kHz and 13 kHz were not significant. *Conclusions:* The presumptive phantom auditory sensation of rats treated with salicylate is most likely different from a pure tone of 8 or 13 kHz and 70 dB SPL. The larger behavioral change for 5 kHz and broadband noise implies that the sound experience of a rat is more similar to a low frequency broadband sound than to a high frequency pure tone.

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Echolocation behavior of *Vespertilio murinus* foraging in open and edge space

368

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The proximity of insect prey to background clutter are the most relevant ecological constraints on foraging bats exerting selective pressure on the design of search signals. Clutter conditions can therefore be used for the definition of bat habitats. Bats forage in open space if they search for prey far from background targets and do not react to such targets in their echolocation behavior. Bats forage in edge space if they search for prey

flying at vegetation edges and in gaps and react to background targets in their echolocation behavior.

In our study we recorded search signals of *Vespertilio murinus* foraging in the open and at vegetation edges and determined signal parameters such as start frequency, peak frequency, terminal frequency, bandwidth and duration for bats flying at different horizontal distances to vegetation and vertical distances to the ground. The bats reacted to background targets only if they flew lower than about 4 m and/or were less than about 6 m away from vegetation. Beyond these borders, i.e. in open space, the signal parameters were not changed in relation to background targets. In open space, bats emitted FM-QCF signals mostly every second or third wing beat with a bandwidth of about 14.3 ± 4.9 kHz and a duration of about 11.7 ± 2.3 ms. Within the measured borders, i.e. in edge space, the bats emitted one search signal at every wing beat. They increased bandwidth und reduced duration with decreasing distance to background targets which resulted in broadband, steeply modulated FM signals.

369 Neural Activation in Auditory Cortical Fields of the Mouse under Anesthetics

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Anesthetics acting on the central nervous system may change response patterns of neurons to sensory stimuli such as sounds. This can lead to difficulties in the interpretation of electrophysiological results taken under anesthesia in comparison with psychophysical, behavioral, and tomographic data in awake subjects. Here, we use c-Fos immunocytochemistry for measurements of activation of mouse auditory cortical neurons under awake and anesthetized conditions to see how the auditory cortex (AC), especially neurons in primary and higher-order fields of the AC [1], respond to different anesthetics.

Female house mice (outbred hybrids of feral Mus musculus domesticus and laboratory mice of the NMRI strain) aged 6-16 weeks were awake or anesthetized by intraperitoneal injections of either a mixture of ketamine (120 mg/kg) and xylazine (5 mg/kg) or Equithesin, which consists of chloral hydrate (176 mg/kg), pentobarbital (40 mg/kg) and magnesiumsulfat (87 mg/kg). The anesthetized animals were sound stimulated after having reached a state without reflexes of toes, tail, and eyelids. Sound stimulation (50 kHz tone bursts of 60 ms duration including 5 ms rise and fall times, 45 ms interburst intervals, 70-75 dB sound pressure level) was done in darkness in a sound-proof chamber. After 15 minutes of continuous stimulation, the sound was turned off and the mice remained undisturbed in the room for futher 30 minutes. Then the animals were killed by cervical dislocation, the brains removed and prepared for c-Fos immunocytochemistry [2]. Fos-positive cells were evaluated in frontal sections through the AC.

First results showed the following differences in the number of Fos-positive cells between awake and anesthetized animals: Anesthesia of both kinds, compared to the awake state, led to a decrease of labeled neurons in the ultrasonic field (UF, part of the primary auditory cortex), and to an increase in the anterior auditory field (AAF, also a primary

field). The latter has no specific tuning to ultrasounds [1]. In the second auditory field (AII, higher-order auditory cortex), major differences in the numbers of Fos-positive cells between awake and anesthetized animals were not evident. These results show that Fos-immunocytochemistry is useful to demonstrate influences of anesthetics on neural activation of the AC and to detect differential effects of anesthesia in auditory cortical fields.

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Representation of the biological significance of a mouse call in the auditory cortical fields 370

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Most mammalian vocalizations e.g. human speech, bats' echolocation calls and wriggling calls of mouse pups (Mus domesticus) consist of harmonically structured sounds. Mothers of house mice respond to pup wriggling calls with maternal care, such as likking, nest building and changing nursing position on her litter. Like other complex communication sounds, wriggling calls are recognized by their harmonic structure and the temporal relationship of the frequency components. Synthesized series of three-frequency call models resembling the main formants of natural wriggling calls (3.8 + 7.6 + 11.4 kHz, 100 ms duration, 200 ms intercall intervalls, 4 calls per series) are a necessary and sufficient releaser of maternal behavior [1]. Long lead- or lag-times of the fundamental frequency make the calls unattractive for the mothers (non-recognition) [2].

Here, we study changes in neuronal activation patterns in the auditory cortex (AC) of mothers being stimulated by wriggling call models that are recognized or not. Neuronal activation is quantified via c-Fos immunocytochemistry by counting Fos-positive cells in the auditory cortical field AI (primary auditory field), AAF (anterior auditory field), AII (secondary auditory field) and DP (dorsoposterior field), in frontal sections of the electrophysiologically identified AC of both hemispheres.

Among our results are the following: In AC primary auditory fields AI and AAF, all call models independent of their "meaning" led to Fos-labeling in accordance with the tonotopy. Significant differences in the amount of Fos positive cells occurred in the higher auditory fields AII and DP depending on whether calls were recognized or not. A left-hemisphere dominant labeling was seen in DP of mothers recognizing the calls. So the "meaning" of wriggling calls seems to be represented first in higher order fields of the AC such as the AII and DP and has an influence of the activity balance in the AC of the left and right hemisphere.

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371 Temporal integration of two sequential tones in mouse inferior-colliculus neurons

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The inferior colliculus of the auditory midbrain may be the first level of the ascending auditory pathways where neurons are sensitive to sound duration and to gaps in streams of auditory objects. As shown in psychoacoustical tests, this time-related information is important for the perception of auditory streams such as human speech and bird song. Coding strategies for either separating or linking objects for auditory perception are not understood.

Here we use extracellular *in vivo* single-unit recordings from the central nucleus of the inferior colliculus (ICC) of the house mouse (outbred hybrid females between feral house mice, *Mus musculus domesticus*, and NMRI outbred strain) under Ketavet/Rompun anesthesia to test the influence of a probe-tone on the response to a following tone at the characteristic frequency (CF-tone) of the studied neuron. Critical variables are the frequency of the probe-tone and the time interval between the end of the probe-tone and the start of the CF-tone. The probe-tone frequency may be placed inside (excitatory or inhibitory actions expected) or outside (facilitatory or no effects expected) the neuron's receptive field, as measured by responses to two simultaneous tones (one probe-tone, one CF-tone; see Egorova et al., 2001. Exp.Brain Res. 140: 145-161).

We show that the probe-tone can have excitatory, facilitatory, inhibitory or no influence on the spike-rate and latency response to the CF-tone. The type of influence can switch depending on the gap between the two tones. Critical time intervals for switching, e.g. from an inhibitory to an excitatory influence, are between 0 ms and 10 ms, 10 ms and 50 ms, and > 100 ms. Thus, neurons in the ICC can code for different time intervals between acoustic stimuli by switching from one response type to another at individually preferred time intervals. This type of sensitivity in the time domain may be responsible for separating or linking acoustic objects in a stream of sounds.

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372 Discrimination and localization of overlapping water surface waves in the clawed frog, *Xenopus laevis laevis*.

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The clawed frog, *Xenopus*, is a fully aquatic frog that retains its lateral-line system beyond metamorphosis. By means of this system it can localise and distinguish water surface waves that simultaneously impinge and overlap on its body (Elepfandt, A.; In Coombs et al. 'Neurobiology and Evolution of the Lateral Line System'. Springer 1989, p. 527). Localisation is possible for at least three simultaneous waves. Since every lateral-line organ only encodes the wave superposition pattern at its specific location on the

animal, discrimination of overlapping waves requires central nervous comparison of the encoded superposition patterns from several organs in order to determine the direction and frequency of the component waves. We have measured, in *Xenopus laevis laevis*, frequency discrimination limens for two overlapping waves. For this, we presented the frog with waves of different frequencies impinging simultaneously from 45° left and right, respectively, and conditioned them to respond only to one of the two frequencies. Variation of wave amplitudes and interchanging the sides of the frequencies guaranteed that discrimination was only possible on the basis of wave frequency. Frequency differences were stepwise reduced until the threshold was reached. For frequencies above 10 Hz, discrimination acuity was better than frequency discrimination of waves that were presented separately. Relative discrimination was best at 18 Hz, where a frequency difference of 0.5 Hz could be recognised. This means that the original waves are computed by the animal from beats with 2 sec cycle length.

When the interstimulus angle was reduced, discrimination acuity deteriorated. At interstimulus angles of 60° and 40° rather than the original 90°, the wave discrimination limens at 18 Hz rose to 0.85 Hz and 1.72 Hz, respectively. At interstimulus angles less than 40°, the animal did not turn to one of the waves, and several features of the frogs' behaviour suggested that 40° was the minimal angle at which *Xenopus* could discriminate waves.

Localisation accuracy under two-wave conditions was not obviously inferior to localisation of single waves. However, at an interstimulus angle of only 40°, that is at the directional limit of discrimination, turn angles tended to be slightly larger than the stimulus angles, as if the animal turned not only toward the correct stimulus but also away from the wrong stimulus, a phenomenon known as peak shift of stimulus discrimination.

Selective loss of Calretinin-immunopositive bipolar neurons in Scarpa's ganglion of vestibular mutant mice 373

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The primary vestibular afferents (PVA) of bipolar neurons (BN) in the vestibular ganglion (VG) connect the peripheral vestibular hair cell sensors of the labyrinth with the vestibular nuclei and the cerebellum in the CNS. Thus, damage to PVA may cause symptoms characteristic for central as well as for peripheral vestibular disorders. BNs and PVA have been shown to outlive extensive damage or loss of vestibular end organs as well as central target deprivation for a long time and in many instances. In cerebellar and vestibular mutant mice vestibular symptoms are evoked by peripheral and central vestibular dysfunction or cell loss, respectively. However, the impact of these disturbances on the maintenance of BPs in these mutants is not known.

In the present study, the total number of BNs was estimated in the VG of the cerebellar mutants *weaver (wv/wv)*, *Lurcher (Lc/+)* and *purkinje cell degeneration (pcd/pcd)* and in the vestibular mutants *jerker (je/je)*, *shaker (sh/sh)* and *Varitint-waddler (Va/+)* using unbiased stereological methods. Moreover, the total number of BNs expressing the

calcium-binding protein Calretinin (Calr), which is a selective marker of a subpopulation of large-diameter PVA with calyx-endings, irregular activity and phasic response dynamics, was quantified by combined physical disector stereology and Calr-immunocytochemistry on semithin sections.

In result, the total number of BNs in the VG of wild-types was 3431 (± 125.19) and the number of Calr-positive BNs was 508 (± 38.98), corresponding to about 15% of the total BN population. Although the complete number of BNs was neither reduced significantly in cerebellar nor in vestibular mutants, the number of Calr-positive BNs showed a clear reduction by 23% in *je/je* and 34% in *sh/sh*. Both, *je/je* and *sh/sh*, showed an additional decrease in cell size and ganglionic volume. The significant reduction of Calr-positive BNs in *je/je* and *sh/sh* might result from a true cell loss, which, due to the small size of this subpopulation, does not reach the level of significancy with respect to the complete population of all BNs. Alternatively, the number of Calr-positive BNs is decreased as a consequence of a downregulation of Calr-expression in these neurons. As in both, *je/je* and *sh/sh*, the mutations affect actin-bundling in stereocilia of hair cells, sensory transduction is likely to be disturbed in these mutants, and this in turn might lead to a reduced hair cell input on PVA. In consequence, there is no further need for a high calcium-buffering capacity, reflected by high level expression of Calr, leading to a decrease in Calr-content beyond immunocytochemical detectability in some of these neurons.

These results not only support the view, that BNs are highly resistant to central target deprivation and peripheral vestibular damage, but also indicate that more subtle alterations in the protein composition of relatively few BNs or in the maintenance of specific BNs may contribute to the vestibular symptoms in these mutants.

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374 Coding of lateral-line stimuli in the goldfish midbrain in still- and running water

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Fish use the mechanosensory lateral line to detect weak water motions. The smallest unit of the lateral line is the neuromast, a sensory structure located freestanding on the skin (superficial neuromasts) or in lateral line canals (canal neuromasts). Recordings from afferents of the posterior lateral line nerve have shown that superficial neuromasts, but not canal neuromasts, are sensitive to unidirectional water flow (Engelmann et al. 2002). This functional subdivision of the peripheral lateral line is maintained to some degree up to the level of the brainstem (Kröther et al. 2002). We investigated whether unidirectional water flow alters the response properties of toral lateral line units.

Up to three units were recorded simultaneously in the right (contralateral) torus semicircularis of the goldfish, *Carassius auratus* (N = 10), using an array of 2 x 3 platinum tungsten electrodes (distance between neighbouring electrodes was 250 µm) mounted in a Reitboeck microdrive (Eckhornsystem, Thomas Recording). Units were stimulated with a sphere (diameter 10 mm) which either was vibrated at a certain position close to the skin of the fish (displacement amplitude 1000 µm, vibration frequency 50 or 100

Hz) or which was moved with a velocity of 15 cm/s from anterior-to-posterior or from posterior-to-anterior along the side of the fish. Sphere stimuli were presented in still- and running (10 cm/s) water.

A total of 33 unimodal lateral line units were encountered in the torus semicircularis of goldfish. Out of these 33 units, 11 were flow sensitive (two units responded with a decrease and 9 with an increase in discharge rate) and 22 were flow insensitive. In still water, 32 of the 33 units responded to the moving sphere. Responses consisted of an increase in discharge rate. Unidirectional water flow reduced the responses of these units, irrespective of whether the units were flow-sensitive or not.

Seventeen of the 32 units which responded to the moving sphere also responded to the stationary vibrating sphere. The vibrating sphere responses of 13 units were masked in running water. The responses of two units were not altered by unidirectional water flow, provided the sphere was placed at a caudal position. If the sphere was positioned more rostral the responses were masked.

The finding that the responses of both, flow-sensitive and flow-insensitive toral units were masked by unidirectional water flow suggests that information arising from superficial and canal neuromasts is combined at the level of the torus semicircularis.

As in a previous study (Plachta et al., submitted) we found a crude somatotopy in the torus semicircularis of goldfish: The receptive fields of the units encountered in the rostral torus were located more rostrally than the receptive fields of the units located in the caudal torus. This somatotopic organization was not restricted to flow-insensitive units.

Engelmann et al. 2002, J Comp Physiol A 188: 513-526. Kröther et al. 2002, J Exp Biol 205: 1471-1484.
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Neural responses of goldfish lateral line fibres to vortex-ring stimuli

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Fish use the mechanosensory lateral line to detect weak water motions. The smallest unit of the lateral line is the neuromast, a sensory structure located freestanding on the skin (superficial neuromast) or in lateral line canals (canal neuromast). Recordings from afferents of the posterior lateral line nerve have shown that superficial neuromasts, but not canal neuromasts, are sensitive to unidirectional water flow (Engelmann et al. 2002). Among natural lateral line stimuli are the water motions in the vortex trail of a swimming fish or the vortex motions downstream of objects situated in water currents (Bleckmann 1994). That fish can detect vortex rings has been shown (Tou 1991).

We analyzed the responses of 34 posterior lateral line nerve fibers of the goldfish, *Carassius auratus* (N = 9), to vortex rings. To do so, we stimulated immobilized fish with vortex-rings moving along the side of the fish. The distance between the place where the vortex rings were generated and the location of the neuromast recorded from was adjusted to 5 cm. The propagation velocity and the duration of the generated vortex rings could be regulated with a computer-controlled valve-bank. During an experiment water

motions were visualized with particle-image velocimetry (PIV). The light sheat in which particle movements were monitored was placed parallel to the ipsilateral side of the fish at a distance of approximately 3 mm. A vibrating sphere (vibration frequency 50 Hz, vibration amplitude 0.02 - 0.6 mm) was used to determine the receptive field of the lateral line units encountered.

Based on discharge rates, two response types could be distinguished: (i) afferents (n = 12) which responded with a transient decrease in discharge rate followed by an increase in discharge rate and (ii), afferents (n = 22) which responded with the inverse discharge pattern. In all cases, response onsets and initial response patterns to successive vortex stimuli were reproducible. Besides the initial well-defined response component, the majority (n = 30) of units showed late response components which were ill defined. To find out whether units received input from superficial or canal neuromasts, we stimulated the lateral line with unidirectional water flow (Engelmann et al. 2002). In addition we stimulated the lateral line with a sphere moving along the side of the fish. We neither found a correlation between the response patterns caused by the moving sphere (e.g. inhibition-excitation-inhibition) and the response patterns caused by the vortex rings, nor did we find a correlation between the sensitivity of a unit to unidirectional water flow and its response pattern caused by vortex rings. In addition, the reproducible and the non-reproducible response components to the vortex ring stimuli were compared with the water motions - analysed with the PIV-system - at the position of the neuromast recorded from.

Bleckmann (1994) Reception of hydrodynamic stimuli in aquatic and semiaquatic animals. Fischer, Stuttgart.
Engelmann et al. (2002) J Comp Physiol A 188: 513-526.
Tou (1991) J Environ Sci Health A26, 755-775.
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376 Responses of lateral line brainstem units to moving objects of different size

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Primary afferent fibers of the fish lateral line project to the brainstem medial octavolateralis nucleus (MON). In running water, flow-sensitive MON units increase or decrease ongoing discharge rate suggesting that they receive input predominantly from afferent fibers innervating superficial neuromasts. In contrast, the activity of flow-insensitive MON units is not affected by running water suggesting that these units process input predominantly from fibers innervating canal neuromasts (Engelmann et al. 2002, Kröther et al. 2002). We wanted to know in which way MON units respond to moving objects and whether flow-sensitive and flow-insensitive MON units represent object size differentially. Therefore, we recorded the responses of MON units in the goldfish, *Carassius auratus*, to objects of different size (7 cm rods, crossections 0.5x0.5, 1x1, 1.5x1.5, 2x2 or 3x3 cm) that were moved in still water along the side of the fish. As in former studies, we used DC water flow to distinguish between flow-sensitive and flow-insensitive MON neurons. As expected (Mogdans et al. 1997) responses to the moving object were highly variable from one unit to the next. To date, 39 units were recorded that responded with one or more peaks of excitation separated by periods of reduced activity while the object passed the fish. Most units (n=36, 92%) exhibited a change in

discharge rate immediately after object motion started, i. e., at a time when the object was still about 15 cm away from the fish. 25 of these units continued to fire either with bursts of spikes (n=12) or with an increased (n=11) or decreased (n=2) rate after the object had passed the fish and even after object motion had stopped. In 11 units discharge rates returned to pre-stimulus levels immediately after the object had passed the fish. Three units were recorded that responded to the moving object only while it passed alongside the fish. In general, with increasing object size response rates increased and response peaks became more distinct. Of the 39 units, 15 (38%) were sensitive to water flow, i. e., they responded to running water with a transient or sustained change in discharge rate, and 24 (62%) were not sensitive to water flow. Systematic relationships between unit sensitivity to water flow and the patterns, strengths or durations of the responses to different object sizes were not apparent suggesting that flow-sensitive and flow-insensitive MON units do not respond differentially to object size. An exception were those units that discharged bursts of spikes after the object had passed the fish. With increasing object size these bursts became stronger and more predictable across stimulus presentations suggesting that object size is represented by the responses of these units to the objects' wake.

Engelmann, Bleckmann, Hanke (2002) J Comp Physiol A 188: 513 Kröther, Mogdans, Bleckmann (2002) J Exp Biol 205: 1471 Mogdans, Bleckmann, Menger (1997) Brain Behav Evol 50: 261
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Responses of superficial and canal neuromasts to moving objects of different size

377

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The lateral line of fishes is comprised of numerous individual neuromasts which occur freestanding on the surface of the skin or in subepidermal canals. Afferent fibers in the posterior lateral line nerve (PLLN) can be distinguished by their sensitivity to running water. Fibers that innervate superficial neuromasts increase ongoing discharge rate in running water whereas the activity of fibers innervating canal neuromasts is not affected (Engelmann et al. 2002). PLLN fibers respond to a passing object with excitation followed by inhibition or vice versa. Fibers innervating superficial neuromasts, in addition, may discharge bursts of spikes even after the object had passed the fish (Mogdans and Bleckmann 1998).

We wanted to know whether fibers that innervate superficial and canal neuromasts represent object size differentially. We therefore recorded the responses of PLLN fibers of goldfish, *Carassius auratus*, to objects of different size (7 cm rods, cross-sections 0.5x0.5, 1x1, 1.5x1.5, 2x2 or 3x3 cm) that were moved in still water along the side of the fish. As in former studies, we used DC water flow to distinguish between fibers innervating superficial and canal neuromasts.

Out of a total of 93 fibers that responded to a moving object, 67 (72%) were sensitive to water flow, i.e., they responded to running water with a change in ongoing discharge rate and thus most likely innervated superficial neuromasts. About half of these fibers (n=31) exhibited bursts of spikes after the object had passed the fish and even for a long time after the object had come to a stop. The other half of these fibers (n=36) did not

show such bursting activity. 26 of the 93 fibers (28%) were not sensitive to water flow and thus most likely innervated canal neuromasts. None of these fibers showed bursting activity after the object had passed the fish. These data are in agreement with previous data that suggested that PLLN fibers innervating canal neuromasts do not or only weakly respond to the water motions in the object's wake whereas a large proportion of PLLN fibers innervating superficial neuromasts respond with bursts of spikes to the object's wake (Mogdans and Bleckmann 1998).

To determine whether fibers that innervated superficial or canal neuromasts responded differently to object size, we determined maximum spike frequency and width of the main response peak that occurred while the object passed alongside the fish. In most units, maximum spike frequency was saturated for all except the smallest object tested. In contrast, the width of the main response peak increased with increasing object size. However, these parameters did not reveal differences between flow-sensitive and flow-insensitive fibers. Thus, based on this analysis, fibers innervating superficial neuromasts or canal neuromasts, respectively, did not encode object size differentially. Whether responses of PLLN fibers to the wake caused by a moving object contain information about object size remains to be analyzed.

Engelmann, Bleckmann, Hanke (2002) J Comp Physiol A 188: 513
Mogdans and Bleckmann (1998) J Comp Physiol A 182: 659
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378 Minimal Model of Prey Localization through the Lateral-Line System

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The clawed frog *Xenopus* is a fully aquatic predator catching prey at night by detecting water movements originating from its prey. To this end *Xenopus* uses its lateral-line system, a mechanoreceptive system found in aquatic amphibians and fish and analyzing water movements along the animal's body. It comprises, dependent on the species, hundreds to several thousands of small lateral-line organs dispersed over the trunk. *Xenopus laevis laevis* has about 180 of them, positioned along several lines at both sides of the body, around the eyes, and at a few other locations of head and neck. A lateral-line organ of *Xenopus* contains 4-8 small cupulae, gelatinous flags protruding into the water and deflected by local water flow. The deflection stimulates sensory hair cells at the base of the cupulae and in this way generates spikes in the lateral-line nerves, phase-locked to the water velocity.

Xenopus' eyes are not adapted to seeing in water and the animal's lateral-line system has become the central sensory organ for spatial orientation. When an insect drops onto the water surface it generates a wave passing along *Xenopus* and stimulating the lateral-line organs. As its natural behavior, the frog will then turn towards the wave's origin, its prey. Not only does the lateral-line system allow *Xenopus* to determine the direction of a single impinging wave but also to resolve the directions of *two simultaneous* waves of different frequency that overlap at the animal, and even to discern wave sources as

"food" and "non-food". Because each lateral-line organ responds to waves from *any* direction, localization requires a *comparison* of inputs from several lateral-line organs. Since, however, each lateral-line organ only encodes the local superposition pattern of the waves at the body surface, the pattern-segmentation ability of *Xenopus* requires both a comparison of the encoded superposition patterns stemming from several organs and a decomposition of the patterns into their original components.

We present a general method, a `minimal model' based on a minimum-variance estimator, to explain prey detection through the frog's many lateral-line organs. The model is robust in the sense that, even if several lateral-line organs are defunct, it still matches experimental data. We show how *waveform reconstruction* allows *Xenopus* to determine both direction and character of the prey and even to distinguish two simultaneous wave sources. A simple neuronal algorithm with realistic firing rates, number of synapses, and time constants of postsynaptic potentials suffices to perform localization and pattern segmentation through waveform reconstruction.

As the model is universal, we expect its results to be applicable to many aquatic amphibians, fish, and reptiles with a large number of local stimulus detectors, such as crocodilians, which have recently been found to take advantage of about 2000 dome pressure receptors at the surface of their face.

The Compensatory Role of Fastigial Vestibular Neurons during Trunk Displacement Relative to Head Position

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Vestibular information is insufficient alone to induce vestibulospinal (VS) reflexes; it requires in addition input originating from neck receptors. The fact that vestibulo- and reticulospinal neurons as well as a large amount of spinal interneurons in animals respond to both whole animal and body-relative-to-head rotation indicates that they receive convergent input from labyrinth and neck receptors. This information converges in the Purkinje cells (PC) of the cerebellar anterior vermis, which is the target of the vestibular nuclei and neck receptors. The anatomy indicates that the PCs affect the VS reflexes via the fastigial nucleus (FN), which innervates the vestibular nuclei. Hence, it was of interest to determine how neck signals are expressed in vestibular neurons of the rostral fastigial nucleus.

We investigated whether stimulation of neck receptors in the alert monkey induced by changing the orientation of the body relative to the head had an influence on the vestibular responses of the fastigial neurons.

The activity of the vestibular neurons was recorded during different frequencies (0.1-1.0 Hz.) while the animal was being rotated in the roll and in the pitch planes under two conditions: (1) with the head aligned with the body and (2) with the body turned by ±45° to either side of the head. The head was always kept immobile while the trunk was positioned by means of an individually modelled corset attached to a rotating platform.

The data so far show that most vestibular FN neurons are influenced by trunk position. In most cases the observed shifts of the neuronal response vector orientation (RVO)

were partly compensatory for the trunk rotation. This indicates that the FN participates in the sensorimotor transformations that account for changes in the relative position of vestibular sensory and effector organs.

380 Does diminished gravity or exclusively zero gravity induce motion sickness in fish?! - A drop-tower experiment -

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It has been repeatedly shown earlier that some fish of a given batch reveal motion sickness (a kinetosis) at the transition from 1g to microgravity. In the course of parabolic aircraft flight experiments, it has been demonstrated that kinetosis susceptibility is correlated with asymmetric inner ear otoliths (i.e., differently weighed statoliths on the right and the left side of the head) or with genetically predispositioned malformed cells within the sensory epithelia of the inner ear. Hitherto, the threshold of gravity for inducing kinetosis as well as the relative importance of asymmetric otoliths versus malformed epithelia for kinetosis susceptibility has yet not been determined.

Therefore, experiments will be carried out at the ZARM drop-tower facility in Bremen, Germany, employing larval cichlid fish (*Oreochromis mossambicus*), which will be kept in a camcorder-equipped centrifuge during the microgravity phases of the experiments and thus receive various gravity environments ranging from 0.1 to 0.9g. Videographed controls will be housed outside of the centrifuge receiving 0g. Based on the video-recordings, animals will be grouped into kinetotically and normally swimming samples. Subsequently, otoliths will be dissected and their size and asymmetry will be measured. Further investigations will focus on the numerical quantification of inner ear supporting and sensory cells as well as on the quantification of inner ear carbonic anhydrase reactivity. A correlation between (1) the results to be obtained concerning the g-loads inducing kinetosis and (2) the corresponding otolith asymmetry/morphology of sensory epithelia/carboanhydrase reactivity will further contribute to the understanding of the origin of kinetosis susceptibility.

381 Effects of vestibular nerve transection on the swimming behaviour and calcium incorporation into inner ear otoliths of fish

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Previous investigations on neonate swordtail fish (*Xiphophorus helleri*) revealed that otolithic calcium incorporation (visualized using the calcium tracer alizarin complexone) and thus otolith growth had ceased after nerve transection, supporting a hypothesis according to which the gravity-dependent otolith growth is regulated neuronally. Subse-

quent investigations on larval cichlid fish (*Oreochromis mossambicus*) yielded contrasting results, repeatedly depending on the particular batch of cichlids investigated. Like most neonate swordtails, Type I cichlids revealed a stop of calcium incorporation after unilateral vestibular nerve transection. Their behaviour after transection was normal, and the otolithic calcium incorporation in controls of the same batch was symmetric. In Type II cichlids, however, vestibular nerve transection had no effect on otolithic calcium incorporation. They behaved kinetotically after transection (this kind of kinetosis was qualitatively similar to the swimming behaviour exhibited by larval cichlids during microgravity in the course of parabolic aircraft flights). The otolithic calcium incorporation in control animals was asymmetric. These results show that the effects of vestibular nerve transection as well as the efficacy of the mechanism, which regulates otolith growth/otolithic calcium incorporation, are - depending on the particular batch of animals - genetically predispositioned. In conclusion, the regulation of otolithic calcium incorporation is guided neuronally, in part via the vestibular nerve and, in part, via a further pathway, which remains to be addressed in the course of future investigations.

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Energy filtering transmission electron microscopy (EFTEM) discloses the site of calcium supply of fish inner ear otoliths

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Inner ear otolith formation in fish is supposed to be performed by the molecular release of proteinacious precursor material from the sensory epithelia, followed by an undirected and diffuse precipitation of calcium carbonate (which is mainly responsible for the functionally important weight of otoliths). Previous experiments have shown that the provision of calcium is regulated by a (likely neuronal) feedback mechanism. The pathway of calcium into the endolymph, however, still remains obscure. Therefore, the presence of calcium within the utricle of larval cichlid fish *Oreochromis mossambicus* was analysed by means of energy filtering transmission electron microscopy (EFTEM). Electron spectroscopic imaging (ESI) and electron energy loss spectra (EELS) revealed discrete calcium precipitations, which were especially numerous in the proximal endolymph (P) as compared to the distal endolymph (D), clearly indicating a decreasing P-D gradient. This finding is in complete agreement with conclusions most recently drawn from physiological experiments. A decreasing proximo-distal gradient was also present within the proximal endolymph between the sensory epithelium and the otolith. Further calcium particles covered the peripheral proteinacious layer of the otolith. They were especially pronounced at the proximal surface of the otolith indicating that otolithic calcium incorporation takes place here, which may explain the decreasing P-D gradient. Other calcium precipitates were found to be accumulated at the macular junctions, which clearly supports an earlier suggestion that the endolymph is supplied with calcium via a paracellular pathway. Overall, the present results strongly suggest that the apical region of the macular epithelium is involved in the release of calcium and that calcium

supply of the otoliths takes place in the proximal endolymph, which is in full agreement with a study employing fluorescent calcium-tracers. (Beier et al., this issue).

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383 Carbonic anhydrase reactivity in inner ear maculae of fish during development under hypergravity

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It has been shown earlier that hypergravity slows down inner ear otolith growth in developing fish. Otolith growth in terms of mineralization mainly depends on the enzyme carbonic anhydrase (CA), which is responsible for the provision of the pH-value necessary for calcium carbonate deposition and thus also is presumed to play a prominent role in Ménière´s disease (a sensory-motor disorder inducing vertigo and kinetosis).

Larval siblings of cichlid fish (*Oreochromis mossambicus*) were subjected to hypergravity (3g, hg; 6hrs) during development and separated into normally and kinetotically swimming individuals following the transfer to 1g (i.e., stopping the centrifuge; kinetotically behaving fish performed spinning movements). Subsequently, CA was histochemically demonstrated in inner ear ionocytes (cells involved in the endolymphatic ion exchange) and enzyme reactivity was determined densitometrically. It was found that both the total macular CA-reactivity as well as the difference in reactivities between the left and the right maculae (asymmetry) were significantly lower (1) in experimental animals as compared to the 1g controls and (2) in normally swimming hg-animals as compared to the kinetotically behaving hg-fish. The results are in complete agreement with earlier studies, according to which hypergravity induces a decrease of otolith growth and the otolithic calcium incorporation (visualized using the calcium-tracer alizarin complexone) of kinetotically swimming hg-fish was higher as compared to normally behaving hyper-g animals. The present study thus strongly supports the concept that a regulatory mechanism, which adjusts otolith size and asymmetry as well as otolithic calcium carbonate incorporation towards the gravity vector, acts via activation/deactivation of macular CA.

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Otolithic calcium uptake in developing fish as visualized by laser scanning microscopy 384

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Since changing the gravity vector (concerning direction and amplitude) strongly affects inner ear otolith growth and otolithic calcium incorporation in developing fish, it was the aim of the present study to locate the site of mineralization in order to gain cues and insights into the provenance of the otoliths inorganic compounds.

Therefore, larval cichlid fish (*Oreochromis mossambicus*) were incubated in the calcium-tracer alizarin complexone (AC; red fluorescence). After maintenance in aquarium water for various periods (1h, 2h, 3h, 6h, 9h, 12h, 1d, 2d, 3d, 5d, 6d, 7d, 15d, 29d, 36d and 87d), the animals were incubated in the calcium-tracer calcein (CAL; green fluorescence). AC thus labeled calcium being incorporated at the beginning of the experiment and would subsequently accompany calcium in the course of a possible dislocation, whereas CAL visualized calcium being deposited right at the end of the test. Subsequently, the otoliths were analysed using a laser scanning microscope, and it was shown that the initial site of calcium incorporation was located directly adjacent to the sensory epithelium and the otolithic membrane. Later, calcium deposits were also found on further regions of the otoliths surface area, where they had been shifted to in the course of dislocation. This finding strongly indicates that the sensory epithelium plays a prominent role in otolithic biomineralization, which is in full agreement with an electron microscopical study (Ibsch et al., this issue).

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Does altered gravity influence the succinate dehydrogenase reactivity in fish vestibular ganglia? 385

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Previous investigations revealed that altered gravity such as hypergravity (hg) effects various adaptation and compensation processes at the level of brain nuclei which are connected to the inner ear by the vestibular nerve. Respective investigations on those neurons, which actually comprise the primary relay stations for vestibular inputs to the brain, i.e., the vestibular ganglia, have hitherto not been undertaken.

We were thus prompted to histochemically determine the reactivity of succinate dehydrogenase (SDH; a key enzyme of the respiratory chain and thus a marker for neuronal activity) in the ganglia utricularis and saccularis (the former transmits linear acceleration such as gravity from the inner ear to the brain, whereas the latter is involved in hearing) as well as (for control) in the diencephalic, non-vestibular Nucleus glomerulosus posterioris (NGp) of fish which had been kept at hg.

SDH-reactivity was histochemically demonstrated and densitometrically analysed on serial sections of entire heads of larval cichlid fish (*Oreochromis mossambicus*), who were kept for 14 or 21days at 3g hg (centrifuge).

It was found that SDH-reactivity within the utricular ganglion was significantly increased in experimental animals as compared to the 1g controls, whereas hg had no effect on SDH-reactivity in the saccular ganglion and in the NGp.

The present results provide a clear indication of the effect of altered gravity exclusively on the metabolic activity/plasticity of a peripheral, vestibular ganglion, which is directly involved in the transmission of gravitationally relevant inputs.

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386 Differentiation of the inner ear of cichlid fish under administration of the ototoxic aminoglycoside gentamicin

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Previous investigations revealed that the growth of fish inner ear otoliths depends on the amplitude and direction of gravity, thus suggesting the existence of a (negative) feedback mechanism. In the course of these experiments, it was shown that altered gravity both affected otolith size (and thus the provision of the proteinacious matrix) as well as the incorporation of calcium. It is hitherto unknown, as of whether sensory hair cells are involved either in the regulation of otolith growth or in the provision of otolithic material (such as protein or inorganic components) or even both. Developing cichlid fish *Oreochromis mossambicus* were therefore immersed in varying concentrations of the ototoxic aminoglycoside gentamicin (GM; 20, 40, 60, 100, 120mg/l; at even higher concentrations, the animals did not survive) for 10 or 21 days. At the beginning and at the end of the experimental periods, the fish were incubated in the calcium-tracer alizarin complexone (AC). After the experiment, otoliths were dissected and the area grown during GM-exposure (i.e., the area enclosed by the two AC labellings) was determined planimetrically. The results showed that incubating the animals in a GM-solution had no effect on otolith growth, but the development of otolith asymmetry was affected. Further examinations, focused on the ultrastructure of the sensory hair cells, revealed, that these cells had not been affected morphologically by GM-treatment. In conclusion, the present results suggest that hair cells are not affected by GM concerning their possible role in (general) otolith growth, but that these cells indeed might have transitionally been effected by GM in terms of a decreased capacity of regulating otolith asymmetry without revealing distinct, degenerative morphological features.

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The natural background noise of electrosensation 387

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Every sensory system has to cope with noise that tends to obscure relevant signals or that may sometimes be read as a signal. To fully appreciate the mechanisms by which a sensory system filters signal from noise we need to determine the statistics of the background noise as experienced by an animal under natural conditions. The electrosensory system of weakly electric fish is an especially appealing study object for this problem, because the electric signals resolved by these animals are exceedingly small. In the context of prey detection, the relevant signals at detection threshold are on the order of a few tenths of a percent of the baseline amplitude of the fish's electric field (Nelson, MacIver (1999) *J Exp Biol* 202:1195-1203). These tiny signals are embedded in a background electric field modulated by the effects of tail bending, of the fish's passing near inanimate objects with an electrical impedance different from that of the water, or by interference with the electric field of a nearby conspecific. Currently, we quantify the statistical properties of noise caused by all of these factors. To do so, we have developed a technique to measure the input signal to the electrosensory system at selected locations on the body of animals swimming freely in a large tank. This input is the potential difference across the skin of the fish, which drives electroreceptors embedded into the skin. The recorded transdermal potential is subjected to standard Fourier and to wavelet analysis. Preliminary data indicate that the amplitude of noise of all the sources mentioned above can be at least an order of magnitude larger than actual prey signals at detection threshold and also that the spectra of noise and prey signal overlap.

Once we have carefully quantified the statistical properties of noise, we will address the physiological mechanisms employed by the early stages of electrosensory processing to filter the behaviorally relevant signals from the unavoidable background noise.

Cellular characterization of neurons constituting the central olfactory pathway of the desert locust *Schistocerca gregaria* by whole-cell soma recordings in an isolated brain 388

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Information processing in the central olfactory pathway of locusts has been studied extensively with intra- and extracellular recordings (e.g. Perez-Orive et al., Science 297: 359-365, 2002). However, these electrophysiological techniques do hardly allow to specifically target the neuron types of interest or to study their cellular properties. To overcome these limitations, we used whole-cell patch-clamp recordings from neuronal

somata in an isolated brain preparation. This new recording technique allows to study voltage- and ligand-activated currents expressed in the somata of specific neuron types and to obtain a detailed morphological characterization of the recorded neurons.

We used the desert locust *Schistocerca gregaria* for our experiments. For the recordings, the brain was dissected from the head, desheathed completely, briefly treated with papain, and mounted to the bottom of a translucent recording chamber connected to a saline flow-through system. On a fixed-stage upright microscope whole-cell recordings from neuronal somata were established under visual control with a patch-pipette filled with an appropriate patch-solution containing 1% biocytin or neurobiotin. After the recording, the brain was fixed and cut on a vibratome in 50 µm thick sections. Biocytin/neurobiotin was visualized with CY3-labeled avidin and the filled neurons were viewed and recorded with a confocal microscope.

Our experiments focused on the neurons constituting the two stages of the central olfactory pathway: the antennal lobes (AL) and the mushroom bodies (MB). We found that the antennal lobes are constituted by two neuron types with distinctive morphological properties and voltage-activated currents, projection neurons (AL-PN) and local interneurons (AL-LN). The mushroom bodies, on the other hand, are constituted by only one neuron type - the Kenyon cells - forming a more homogeneous neuron population. All AL-PNs (N = 32) have dense multiglomerular arborizations in the AL and an axon projecting via a medially located tract to the calyx of the ipsilateral mushroom body and further to the lateral horn. All AL-LNs (N = 7) have fine arborizations that fill the entire neuropil area of the AL but do not delineate its glomerular organization. The voltage-activated currents expressed in the somata consist of fast inward and sustained outward K-currents in all AL-PNs, whereas in the somata of all AL-LNs only sustained outward K-currents are present. Consequently, the somata of AL-PNs are able to generate action potentials when recorded in current clamp, whereas the somata of AL-LNs are not. All Kenyon cells (N = 42) have spiny arborizations in the calyx of the mushroom body, an axon that travels through its pedunculus and bleb-bearing terminals in the α- and β-lobes. All Kenyon cells express fast inward and sustained outward K-currents in their somata. The inward currents consist of distinct Na- and Ca-currents that underlie the generation of two distinct types of action potentials.

We expect more detailed studies of the membrane currents of the 3 neuron types constituting the central olfactory pathway of locusts will significantly further our understanding of their electrophysiological properties that underlie olfactory information processing

389 Antennal sucrose perception in the honey bee

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Sucrose perception is an important determinant in honey bee foraging behaviour and learning (Page and Erber, 2002). Therefore, antennal sucrose perception in the honey bee was analysed using behavioural, electrophysiological, and pharmacological methods.

Proboscis extension occurs at median latencies of 320-340ms after stimulation of the antennal tip with sucrose. The behavioural response latencies are 80-100ms longer than the latencies measured in the M17 muscle which controls proboscis extension. When bees can actively touch a sucrose droplet with one antenna, they contact the solution frequently for time periods of 10-20ms with mean intervals between contacts of approximately 40ms. Contact duration is negatively correlated with sucrose concentration of the stimulus, while the number of contacts is positively correlated with concentration.

Electrophysiological recordings show that taste hairs of the antennal tip are responsive to sucrose concentrations of at least 0.1%, demonstrating a higher sensitivity than taste hairs of the proboscis. Response magnitude shows a sigmoid dependency on sucrose concentration, reaching saturation at a concentration of 3%. Sucrose responses between different hairs even in the same individual show a high degee of variability, ranging from 1 spike to over 40 spikes for a stimulus of 0.1%.

Injections of the biogenic amines octopamine and tyramine into the head capsule at quantities of 10fmol and 10pmol have only small effects on the response magnitude of taste hairs. The octopamine antagonist epinastine has a strong suppressive effect if 10pmol are applied. Large doses of octopamine significantly elevate the transepithelial voltage accross sensilla without detectable impact on stimulus-induced spike activity.

We conculde that sensitivity of gustatory hairs on the antenna can be modulated by biogenic amine receptor ligands. Antennal scanning behaviour is dependent on the concentration of the sucrose stimulus. As a consequence of short contact durations with a sucrose stimulus, bees primarily use the early phase of sensory cell responses.

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Odor induced activity patterns in the antennal lobe of *Drosophila melanogaster* 390

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The first brain structure involved in odor processing in insects is the antennal lobe, which is the functional and structural analogue of the vertebrate olfactory bulb. In the antennal lobe, sensory input from olfactory sensory neurons (OSNs) terminates in glomerular structures. For some insects it has been shown that local computation accomplished by interneurons shapes the response patterns of the output neurons, the olfactory projection neurons (PNs). In order to understand the information processing in this network, it is necessary to separately record odor-evoked activity from the different neuron populations involved. In a first set of experiments we have studied the activity patterns elicited by odors in PNs of *D. melanogaster* using optical imaging methods. We took advantage of a recently developed technique (Fiala et al., 2002) and expressed the genetically encoded calcium sensor Cameleon 2.1 using the UAS-GAL4 system with the projection-neuron selective line GH146.

We have screened the responses to a total of 11 pure odors, each across a concentration range spanning 8 log units. The applied odors can be divided in two groups: one functional group (aldehydes) with systematically increasing chain length (pentanal, hexanal, heptanal, octanal and nonanal) and odors with different functional groups commonly used in behavioral experiments or of reported relevance for fruit flies (benzaldehyde, butanol, octanol, isoamyl acetate, ethyl acetate and propionic acid).

We found that:

- Different odours induced responses in different areas of the antennal lobe

- The areas activated by specific odors appear to be conserved across animals

- In general, increasing concentrations of one odor elicited increased responses in a given area and also broadened the area of response.

As an example, the aldehydes pentanal, hexanal and heptanal (C5, C6 and C7) elicited responses of similar intensity in the dorso-medial area of the antennal lobe (DM glomeruli), but also in more lateral areas. Increasing concentrations of these aldehydes elicited responses of increasing amplitude. Further increasing the carbon chain length resulted in diminished, but still concentration-dependent responses for octanal (C8), and finally no responses at all for nonanal (C9).

These data confirm that odour representation in the antennal lobe of the fruit flies follows a combinatorial logic, and that both odour quality and stimulus concentration are represented in the glomerular activity patterns of the antennal lobe. *D. melanogaster* thus appears as an ideal model animal to study the mechanisms of olfactory processing in the brain.

391 Olfactory responses database of functional calcium imaging data recorded from the antennal lobe of the honeybee *Apis mellifera*

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Olfactory glomeruli are a common theme of the first brain areas processing odours, be it the insect antennal lobes or the mammalian olfactory bulbs. When an animal smells an odour, a characteristic pattern of glomeruli is activated, suggesting a combinatorial logic to the olfactory code. Functional imaging studies have measured such patterns in a variety of animals, including mice, zebrafish and insects. A major advantage of the insect system is the possibility of morphologically identifying individual glomeruli. This tool allows to compare data between animals. We found that individual glomeruli have characteristic odour-response profiles. Knowing these profiles is necessary to investigate the basic rules of the olfactory code. However, each in-vivo calcium imaging measurement yields a considerable amount of data: first, the physiological data itself consists, for each odour, of many glomeruli activated to differing degrees, second, accessory data such as time-of-day, seasonal influences, age of the animal, odour testing sequence and

other parameters may all be relevant and have an effect upon the response patterns. Developing efficient tools that allow to search for such effects is therefore of great importance.

Our lab has accumulated physiological responses from the honeybee, *Apis mellifera*, over several years. We are now developing a database system which will allow accessing these data via a user-friendly interface. The database will be freely available to the scientific community, and will give access both to the physiological data itself (identified glomerulus, odour presented, time-course of the response) as well as to relevant accessory data. We hope that this tool will prove valuable to theoretical neuroscientists investigating the olfactory code, and that it will serve for comparative purposes involving species other than the honeybee.

A deeper insight: *in vivo* imaging olfactory glomeruli deep inside the antennal lobe of the honeybee using 2-photon scanning microscopy 392

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Preceding calcium imaging experiments featuring the first olfactory neuropil of insects, the Antennal Lobe (AL), have been restricted to a dorsal proportion of the spherical structure. Thus only a part (~25%) of the olfactory processing units, the glomeruli, could be measured. This is unsatisfying, especially because we know from electrophysiological work that two major glomerular sub-populations show differences at the output-level, but only one of these has been accessible for imaging experiments thus far. Many of the sideward glomeruli receive ORN-output through a distinct tract, the antennoglomerularis tract 3 (T3), while the great majority of dorsal glomeruli are fed by ORNs projecting through T1. At the glomerular output level, the projection neurons (PNs) from T1 and T3 glomeruli descend through two distinct tracts, the medial antennocerebralis tract (mACT, T3-glomeruli) and the lateral ACT (lACT, T1-glomeruli). In this study 2-Photon excitation of specifically stained output neurons of the AL has been used to access both populations of glomeruli. We were able to stain the two PN-populations independently, focus deeper into the tissue, cause less photo damage, and resolve structural details much better than in preceding calcium imaging studies. Preliminary results show that *in vivo* imaging of glomeruli deep inside the tissue is feasible. First results do not imply a fundamental difference between responses from T1- and T3-glomeruli, but extracellular recordings performed at our lab indicate that the differences may be visible only at a faster timescale. Ongoing developments aim to increase the temporal resolution of the measurements.

393 Functional response spectrum of genetically identified olfactory sensory neurons in the fruit fly *Drosophila melanogaster*

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Olfactory sensory neurons (OSNs) provide an animal with information about odors in its environment. In mammals and insects, individual OSNs are likely to express only a single olfactory receptor (OR). OSNs expressing the same OR gene converge onto one or a few spatially invariant glomeruli in the olfactory bulb (mammals) or antennal lobe (AL, insects), the first olfactory processing center in the brain. About 60 OR genes have been identified in *D. melanogaster* to date. However, only little is known about which odors activate a particular receptor.

In order to fill this gap, we are currently characterizing the response spectrum of OSN populations expressing the same OR gene in great detail. Using the *Drosophila* UAS-GAL4 system, we create flies expressing Cameleon, a calcium sensitive fluorescent dye, under the control of a particular OR gene promotor. This allows us to monitor the odor-evoked responses of the OSNs expressing this OR gene *in vivo* by using optical imaging. We have tested a panel of 93 odors from a variety of chemical groups. We are measuring odor evoked responses from the primary dendrites and somata of the OSNs on the antenna, and from their terminals in the glomeruli of the AL. The receptor Or22a had a very broad response spectrum: it responded to a third of the odors tested, a large number of which, like ethyl butanoate or isoamyl acetate, can be found in banana extract. Comparing the response spectrum measured in the AL to that measured on the antenna will allow us to see whether the olfactory response profile in the AL has been modified with respect to the primary response in the OSN dendrites.

Knowledge about the response spectrum of the OSNs is crucial for understanding the mechanisms of olfactory coding.

394 Multichannel Recordings in the Antennal Lobe of the Honeybee Suggest Mechanisms of Olfactory Coding via Neuronal Ensembles.

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Olfactory coding in the insect antennal lobe has been related on the one hand to a spatially distributed, combinatorial activity pattern of glomeruli and projection neurons, and on the other hand to a temporal coding of fluctuating subsets of projection neurons with coincident spike activities.

Evidence for the spatial code comes from Ca-Imaging of glomerular activities and from single neuron recordings combined with intracellular marking. The temporal coding hypothesis is supported by intracellular recordings combined with local field potential recordings, and from extra cellular multi-unit recordings. The relationship between spatial and temporal aspects of olfactory coding is unknown.

In the honeybee brain approximately 800 projection neurons connect the antennal lobe with the mushroom body via two main tracks of projection neurons, the l-ACT and m-ACT. Neurons in the two tracts respond to odors with characteristic response patterns indicating an across-fiber-spatial code combined with odor specific patterns (Müller, Menzel, J Comp Physiol A 2002). It is thus likely that both spatial and temporal components contribute to the olfactory code in projection neurons. However, it is unknown whether the coincident activity occurs between neurons of the same or different tract, and during which part of their odor response.

We use multi-channel silicon microprobe arrays (Michigan Center for Neural Communication Technology sponsored by NIH NCRR grant P41-RR09754) to record multi-unit-action and local field potentials simultaneously. With this technique we are able to record neural population activity of antennal lobe projection neurons with a temporal resolution equal to intracellular recordings.

The recorded odor induced neural spikes range between 130 – 400 µV p-p which can be well distinguished from muscular spikes (150 – 600 µV p-p) and biological background noise (80-130 µV p-p). Moreover, we observe units which respond to single or multiple odors, respectively, and show the former described response patterns of projection neurons. Stimulating with one odor several times we observe units changing their temporal response pattern.

Applying automatic and manual spike sorting techniques we accomplish so far to separate up to 5 units within one recording. Further analysis will reveal how spatial and temporal coding schemes interact at the level of projection neurons.

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Neural Dynamics and Odor Coding: Is the Olfactory System a Support-Vector Machine? 395

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The neural dynamics in the antennal lobe of the Honeybee is studied during stimulation with odors. The response of this neural network to each odor is represented as a trajectory in a multidimensional space, where each dimension accounts for the activity of a single glomerulus measured with calcium imaging. The velocity and acceleration of the trajectories are then calculated. It turns out that each trajectory reaches a steady state (fix-point attractor) after over half a second. Furthermore it is shown by means of a support-vector machine that the attractors are odor specific and reproducible across individuals. Our results support the hypothesis of a spatial olfactory code. Combining

these findings with the anatomy we sketch a readout mechanism for a network next to the antennal lobe.

Key words: neural dynamics, support-vector machine, olfactory code, antennal lobe, calcium imaging

396 A gustatory receptor in carbon dioxide sensitive olfactory neurons of *Drosophila*

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Olfaction in *Drosophila* employs ca. 1300 olfactory receptor neurons (ORNs), which can be divided into different classes depending on their response spectra. Each class is thought to express a different member of a family of olfactory receptors (OR). In addition a few gustatory receptors (GR) are also expressed in antenna, one of which is called *Gr21a* (Scott et al., 2001). Recognition and discrimination of odorants most likely involves combinatorial coding. Alternatively, certain odors may have a special signaling function and be encoded via 'labelled lines' with very specific response properties.

In antennal basiconic sensilla 22 classes of ORNs with different response spectra have been described (de Bruyne et al., 2001). Most respond to several odorants but we identified one neuron class (ab1C) that responds exclusively to CO2. These cells are highly sensitive to small fluctuations around ambient concentrations (0,03%). We then used the GAL4-UAS system to drive several reporter genes under control of the *Gr21a* promoter. The expression pattern of a GFP reporter suggests that *Gr21a* is expressed in ab1 sensilla. *Gr21a*-driven expression of the cell-death gene rpr deletes most GFP positive cells. Electroantennograms of such flies show a marked reduction in antennal responses to CO2 but not to other odorants. We also expressed the calcium sensitive cameleon protein in ab1C neurons. In optical recordings on transgenic flies we measured a dose-dependent activation by CO2 but not by other odorants. In addition, the V glomerulus was clearly labeled and showed similar responses. We conclude that ab1C neurons express the gustatory receptor Gr21a.

It is noteworthy that the *Anopheles gambiae* Gr22 receptor shows high homology with *Drosophila Gr21a* (Hill te al. 2002). Mosquitoes are known to use CO2 as a cue in host finding. In a simple orientation assay, *Drosophila* avoids a wide range of CO2 concentrations. The response reaches saturation at a dose well below anesthetic levels that generates 50 spikes/s from ab1C neurons. Such a response may serve to avoid noxious levels of CO2 present in fermenting fruits.

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P2X-receptor expression in cultured rat trigeminal neurons 397

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Trigeminal nerve fibers innervating the facial mucous membranes are sensitive to chemical stimuli with the primary function of protection from harmful chemicals. Additionally, the trigeminal system can mediate odour sensations as shown psychophysically in anosmic humans.

It has been suggested recently, that the neuromodulator ATP modulates odour responsiveness of ORNs by activating purinergic receptors. We investigated the expression of purinergic receptors (P2X) in the trigeminal system by using dissociated cultured neurons of the rat gasserian ganglion. ATP-induced currents were characterized using the patch clamp technique and showed three different time courses of the response: sustained, transient and mixed. We pharmacologically identified three subpopulations of neurons expressing different subtypes of P2X-receptors: Neurons showing sustained currents likely express homomeric P2X2 receptors, the subpopulation showing transient currents expresses homomeric P2X3 receptors. The mixed current was probably based on the expression of two different populations of homomeric P2X2 and P2X3 receptors.

Immunostaining with subunitspecific antibodies supported the pharmacological data and revealed expression of P2X receptors on the somata and fibres. The functionality of these receptors located on the trigeminal fibres could be shown in Ca-Imaging experiments by local ATP-superfusion.

The role of the purinergic receptors in the perception of chemical stimuli like odorants is still under investigation.

Differential expression of odorant receptor mRNA in rat tissues 398

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Odorant receptors are a multigene family encoding seven-transmembrane-domain receptors and belong to the superfamily of G-protein coupled receptors. Approximately 1000 different odorant receptors are thought to exist within the rat olfactory epithelium, distributed in specific zones of the olfactory sheet. Some odorant-like receptors are found in the accessory system, the vomeronasal organ and few receptors are described in testis and sperms. The question arouse if odorant receptors were also expressed in other non-neuronal tissues. We did a systematic analysis of the expression of odorant receptor mRNAs in tissues of the rat (olfactory mucosa, olfactory bulb, cerebral cortex, eye, adrenal gland, kidney, heart, skeletal muscle, intestine, lung, liver, testis) by using RT-

PCR and the appropriate primers. The primers were designed to recognize specifically one type of odorant receptors (OR-F2, F3, F5, F6, F12, I3, I7, I8, I9, I14, I15; OR-nomenclature according to Buck & Axel, 1991). All mRNAs could be demonstrated in the olfactory epithelium and olfactory bulb, additionally some odorant receptors were expressed in several non-olfactory tissues with a differential expression pattern among olfactory receptors and across tissues. Several mRNAs were found in liver, partially the same and others in kidney, testis, brain and the other non-olfactory tissues. None of the investigated olfactory receptor mRNAs was found in skeletal muscle. Each odorant receptor is capable of interacting with one or a small number of ligands to detect odor signals. The expression of odorant receptors in non-olfactory tissues raises the question of their function in those tissues. Odorant receptors are seven-transmembrane-domain proteins, a structural characteristic that is also seen in other "chemo" receptors such as neurotransmitter and hormonal receptors. Dependent on the linkage to the signal cascade of G-proteins or other second messengers within the cell, the odorant receptors in non-olfactory tissues might act as such chemosensitive proteins probably detecting metabolic substances.

399 Efficiency and modulation of spike-evoked calcium influx into olfactory bulb granule cells

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In the mammalian olfactory bulb, axonless granule cells mediate lateral inhibitory interactions between mitral cells via a dendrodendritic reciprocal synapse. What specializations of calcium signalling occur in a cell type in which dendritic spines are the locus of both synaptic input and transmitter release? We addressed this question by examining calcium transients in granule cells using two-photon microscopy in rat brain slices. Somatically evoked action potentials (APs) produced calcium transients that propagated throughout the dendritic tree with amplitudes increasing with distance from soma and attaining a plateau level within the external plexiform layer (EPL), the zone of synaptic output. Interestingly, the degree of boosting correlated with the distance of the soma from the EPL, suggesting a "democracy" of output amongst granule cells.

On average, AP-evoked calcium transient amplitudes were equal in size in spines and adjacent dendrites. They were strongly regulated by the pre-spike membrane potential, linearly decreasing with depolarization and increasing with hyperpolarization. At room temperature, this voltage-dependence was saturated after less than 500 ms of polarization. It was blocked by the application of Ni^{2+} or mibefradil, indicating the involvement of T-type calcium channels. Mibefradil also reduced AP-mediated synaptic transmission from granule to mitral cells. These observations suggest novel mechanisms for regulation of lateral inhibition by granule cells. Strong coincident input will produce a global dendritic release whose magnitude is regulated by the level of sustained input. The voltage-dependent conductance may also interact with the respiration-related slow oscillatory activity prominent in the olfactory bulb.

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Voltage-sensitive dye imaging of odor-evoked oscillatory activity in the zebrafish olfactory bulb

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Odors evoke local field potential (LFP) oscillations in the olfactory bulb (OB) that arise from feedback between the principal neurons, the mitral cells (MCs), and local inhibitory interneurons, the granule cells (GCs). In response to an odor, a subset of MCs lock their spike output to the LFP oscillation. Thus, oscillatory network activity influences the timing of MCs spikes, which may play important roles in olfactory coding.

Classical LFP recordings reveal little about the spatial properties of population odor responses. Here we used voltage sensitive dyes to optically record spatio-temporal activity patterns in the zebrafish OB in response to amino acid and food odors. Signals were detected with a fast CCD camera at a frame rate of 1 kHz. Odors evoked complex spatio-temporal optical signals, including a robust 20 to 30 Hz oscillation that corresponded to simultaneously recorded LFP oscillations.

Odor-evoked oscillations developed gradually after stimulus onset and decreased slightly in frequency as the response proceeded. Oscillatory activity was widely distributed and overlapped substantially for different odors. In most cases, oscillatory activity was not synchronous, but propagated across the OB in a wave-like manner. The average direction and speed of wave propagation was independent of the odor but characteristic for each animal. Thus, oscillations at different locations in the OB are phase-shifted.

A cycle-by-cycle analysis at high resolution revealed rich spatio-temporal structure and high variability of oscillatory activity. The origin, direction, and speed of wave propagation changed during different epochs of the odor response. The distribution of oscillatory power across the OB was highly structured and changed over time during the odor response. In repeated trials with the same stimulus, these wave properties showed a high degree of variability.

These results reveal a complex and dynamic spatio-temporal organization of odor-evoked oscillations in the OB. The variability observed at high spatial resolution suggests that the fine structure of oscillatory activity patterns does not carry significant odor information by itself. We are currently examining the relationship between odor-evoked oscillatory population activity and mitral cell spiking by simultaneous voltage-sensitive dye imaging and single unit recordings. Preliminary results indicate that mitral cell spike timing is controlled by the global, rather than fine, structure of oscillatory activity. This is consistent with the finding that action potentials of distributed subsets of mitral cells synchronize. The synchronization of odor-specific subsets of mitral cells enables the simultaneous representation of multiple features of an odor in a dynamic network (see abstract by Friedrich et al.).

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401 CAMP-independent and cAMP-dependent transduction in olfactory receptor neurons of *Xenopus laevis* tadpoles

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Whether odorants are transduced by only one or more than one second messenger has been a long-standing question in olfactory research. Here we give an answer to this question using a novel preparation, the *Xenopus laevis* tadpole mucosa slice. We show that some olfactory receptor neurons (ORNs) respond to stimulation with amino acids with an increase of the intracellular calcium concentration $[Ca^{2+}]_i$. In order to see whether or not these responses were mediated by the cAMP transduction pathway we applied forskolin or the membrane-permeant cAMP analogue pCPT-cAMP to the olfactory epithelium. The ensemble of ORNs that was activated by amino acids markedly differed from the ensemble of neurons activated by forskolin or pCPT-cAMP. Less than 6% of the responding ORNs showed a response to both amino acids and the pharmacological agents activating the cAMP transduction pathway. We conclude that ORNs of *Xenopus laevis* tadpoles have both cAMP-independent and cAMP-dependent olfactory transduction pathways.

402 CAMP-independent responses of olfactory neurons in *Xenopus laevis* tadpoles and their projection onto olfactory bulb neurons

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We report on responses of olfactory receptor neurons (ORNs) upon application of amino acids and forskolin using a novel slice preparation of the olfactory epithelium of *Xenopus laevis* tadpoles. Responses were measured using the patch-clamp technique. Both amino acids and forskolin proved to be potent stimuli. Interestingly, a number of ORNs that responded to amino acids did not respond to forskolin. This suggests that some amino acids activate transduction pathways other than the well-known cAMP-mediated one.

The differential processing of cAMP-mediated stimuli on one hand and amino acid stimuli on the other was further elucidated by calcium-imaging of olfactory bulb neurons using a novel nose-olfactory bulb preparation of *Xenopus laevis* tadpoles. The projection pattern of amino acid-sensitive ORNs to olfactory bulb neurons differed markedly from the projection pattern of forskolin-sensitive ORNs. Olfactory bulb neurons activated by amino acids were located laterally compared to those activated by forskolin, and only a small proportion responded to both stimuli. The ensemble of neurons activated by forskolin was also activated by IBMX and pCPT-cAMP.

We therefore conclude that sensory transduction of a number of amino acids is cAMP-independent, and amino acid- and cAMP-mediated responses are processed differentially at the level of the olfactory bulb.

Neuronal representation of odourants in the olfactory bulb of *Xenopus laevis* tadpoles 403

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When an odourant enters the nose, olfactory receptor neurons convey information about it to the olfactory bulb, where this information is processed and where the first central representations of the odourant are generated. We show how odourants are represented by ensembles of OB neurons, in particular mitral cells which are the output neurons of the OB. We were able to demonstrate for the first time that the intracellular calcium concentrations ($[Ca^{2+}]_i$) in the somata of these neurons undergo specific changes and that different stimuli are represented by different neuronal ($[Ca^{2+}]_i$) patterns. The similarity of patterns was assessed by cross-correlation analysis. We further show that noradrenaline, which is reported to be involved in olfactory memory formation and to modulate synaptic transmission at dendrodendritic synapses in the OB, profoundly changes the representation of odourants at the level of mitral cells.

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Organization of glomeruli in the olfactory bulb of *Xenopus laevis* tadpoles 404

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The synaptic input layer in primary olfactory centers like the olfactory bulb (OB) in vertebrates or the antennal lobe in insects typically is organized into glomeruli, i.e. spheroidal areas of dense synaptic neuropil. Glomeruli are believed to play a key role in mapping and synaptic processing of olfactory sensory information in the brain. Recent evidence suggests that glomeruli presumably serve as functional units in olfactory coding. However, it is also known that structural features of glomeruli can exhibit a significant degree of variability within the OB of a given species and especially among primary olfactory centers of different species such as lower and higher vertebrates. Differences also exist in the glomerular borders formed by glial cells and/or somata of periglomerular neurons. The functional impact of the heterogeneity in the cytoarchitecture and the periglomerular borders still is unclear. Taken together, an uncertainty remains as to what is meant exactly by the term “olfactory glomerulus”.

Tadpoles of the clawed frog *Xenopus laevis* provide an experimentally favorable model system to study functional and morphological aspects of olfaction both at the peripheral and central levels. Furthermore, development and metamorphosis of the olfactory system of *Xenopus* revealed fundamental aspects of the development of olfactory systems and the nervous system in general. The cytoarchitecture of olfactory glomeruli in pre- and postmetamorphic *Xenopus* was analyzed with special focus on the segregation of individual glomeruli, the cellular basis of heterogenity and the structural basis of glomerular borders at pre-metamorphic stages.

Calcium imaging of odor response patterns of OB neurons revealed that the synapses within the glomeruli are functional in tadpoles. Tracing axons of individual olfactory receptor neurons (ORNs), dendrites of mitral/tufted (M/T) cells and processes of periglomerular interneurons, as well as immunocytochemical labeling of glial cells indicate that the glomerular architecture is solely determined by terminal branches of ORN axons and tufts of M/T primary dendrites. The small population of periglomerular neurons forms wide-field arborizations that always extend over many glomeruli, enter the glomeruli but lack any glomerular tufts. Antibodies to synaptophysin indicate a high density of synapses within glomeruli which was further confirmed at the ultrastructural level and quantified to about 0.5 synaptic sites per μm^2.

Combining immunocytochemistry and ultrastructural investigations we show that glomeruli in *Xenopus* tadpoles lack any cellular borders. In both pre- and postmetamorphic *Xenopus*, glomeruli are surrounded neither by periglomerular somata nor by glial processes. Taken together, our results demonstrate that olfactory glomeruli in *Xenopus laevis* (i) are spheroidal neuropil aggregations of axonal tufts of ORNs and tufts of primary dendrites of M/T cells, and (ii) are not enwrapped by a border of juxtaglomerular cells. Grant sponsor: DFG grants (SFB 406, A12 and B5).

405 Symmetrical nervus terminalis innervation of the retina in asymmetrical fish (Pleuronectiformes)

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An external side asymmetry is evident in flatfish. Even parts of the brain, like the olfactory system are built asymmetrically. Recently, during turbot development the terminal nerve organization was studied with neuropeptide Y (Prego et al. 2002, J Chem Neuroanat 24: 199-209), which cross-reacts with FMRFamide under immunocytochemical conditions. FMRFamide, a molluscan cardioexcitatory peptide exists in a subsystem of the nervus terminalis, which directed from cells of the nucleus olfactoretinalis to the retina. So far no other flatfish species has been studied with respect to the symmetry or asymmetry of this projection. Therefore, FMRFamide immunoreactivity was examined in the retina of a left- and a right-eyed flatfish species. In the left as well as in the right retina of *Scophthalmus maximus* and *Pleuronectes platessa* FMRFamide binded specifically to neurites in the inner nuclear and inner plexiform layer.

In both species, FMRFamide immunoreactivity was found to be distributed almost identically in the left and right retina and likewise in the optic nerve, the nucleus olfacto-

retinalis and in the central target, the optic tectum. This suggests, that even in the periphery this nervus terminalis subsystem perceived chemosensory stimuli that act symmetrically on the asymmetrical fish in contrast to the related main olfactory system. Moreover, in an evolutionary context the symmetry of the olfactoretinalis projection might be termed a plesiomorphic character of the Pleuronectiformes.

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Mixture interactions in the zebrafish olfactory bulb 406

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The perceived quality of an odor mixture is usually distinct from that of its components [1], suggesting that the neural representation of mixtures involves non-linear interactions between the responses to the components. Such interactions include suppression or synergism and may occur at different levels of odor processing [2-5]. In the zebrafish, calcium imaging of afferent glomerular activity in the olfactory bulb (OB) indicates that mixture interactions in the peripheral olfactory system are weak (see abstract by Yaksi et al.). Here we investigated mixture interactions at the next level of processing, the neuronal circuitry of the OB, by electrophysiology and calcium imaging. Odor stimuli used were either single amino acids (10 – 100 μm) or complex food extracts.

Extacellular and whole cell recordings were performed in the outer layer of the ventrolateral zebrafish OB that contains predominantly mitral cells (MC). In some cases, the recorded neurons were loaded with a dye and morphologically identified as MCs. MCs respond to multiple odors with odor-dependent temporal patterns of excitation and inhibition (slow temporal patterning) [7]. In addition, some responses include subthreshold membrane potential oscillations and action potentials phase-locked to odor-evoked oscillatory population activity.

Odor responses of individual neurons to individual stimuli and their binary mixtures were recorded. For amino acid stimuli, the average firing rate and slow temporal pattern of the response to the mixture were usually similar to the response evoked by one of the components. Hence, one component dominated over the other. In mixtures of one predominantly excitatory and one inhibitory component, the excitatory response dominated in 4/19 cases. MC responses of binary amino acid mixtures are therefore largely predictable from the response to the dominant component.

Responses to binary mixtures of food odor stimuli, however, were more distinct from the components' responses. In each epoch, the sign of the response (ecxitation or inhibition) corresponded to at least one of the component responses. The slow temporal pattern and the average firing rate, however, were often different from those of the components' responses. Hence, the response to binary mixtures of complex odors cannot be predicted accurately from the responses to the components.

These results indicate that significant non-linear mixture interactions occur in odor responses of zebrafish MCs. These interactions affect both the firing rate and the slow temporal response pattern. Mixture interactions were significantly stronger for complex

odors that activate many more glomeruli, suggesting that they involve distributed processing. Because only weak mixture interactions were observed in patterns of input activity to the OB (abstract by Yaksi et al.), non-linear mixture interactions must be the result of processing by the OB circuitry.

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407 Spatio-Temporal Dynamics of Receptor Neuron Input to the Mammalian Olfactory Bulb

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Odors evoke dynamic glomerular activity patterns in the olfactory bulb (OB, Spors and Grinvald, 2002). To analyze the dynamics of these patterns at the level of input to the OB, we selectively loaded olfactory receptor neurons with Calcium Green dextran and imaged afferent glomerular calcium dynamics in freely breathing or artificially sniffing, anesthetized mice (Wachowiak and Cohen, 2001). Glomerular odor responses differed in response latency, rise time, decay time, and modulation by sniffing. In response to esters and hydrocarbons, caudo-lateral glomeruli generally exhibited faster responses and more pronounced respiratory modulation. However, neighboring glomeruli could also exhibit different temporal response characteristics. Temporal response characteristics of individual glomeruli depended on glomerulus identity, odor identity, odor concentration, sniffing frequency, and flow rate. Changing from freely breathing to artificially sniffing altered the degree of respiratory response modulation, while differences in response latency, rise time, and amplitude across glomeruli and odors were preserved. The temporal response properties were consistent for equivalent glomeruli in different preparations. Hence, the spatial pattern of glomerular input can change significantly over time in a stimulus-specific manner. The spatio-temporal dynamics of afferent activity patterns therefore need to be considered in models of olfactory coding. Support by BMBF, MPG, NIH DC00378, DC04938, and DC05259.

Different odor information conveyed by synchronous and asynchronous mitral cell firing patterns

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Mitral cells (MCs) are the principal olfactory bulb (OB) neurons and display odor responses that are temporally patterned on at least 2 time scales: (1) over hundreds of ms, MC responses consist of alternating excitatory and inhibitory epochs, and (2) odor-specific subsets of MC oscillate and synchronize their action potentials with millisecond precision. Since individual MCs respond to multiple odorants, odor identity information is contained in the ensemble response. The change of the ensemble response over time results in a decorrelation of initially similar activity patterns that could facilitate odor identification [1]. Here we studied fast oscillatory synchronization across multiple MCs using electrophysiology and voltage-sensitive dye imaging in the OB of zebrafish.

Optical and multisite local field potential (LFP) recordings revealed wide-spread odor-evoked oscillatory activity (frequency, 20 – 30 Hz) that propagated as waves across the OB and probably reflects granule cell activity. On average, wave structure and propagation were independent of odor, but characteristic for each animal. Hence, the phase of the LFP oscillation differs across the OB.

Oscillatory MC firing is locked to the LFP. However, the preferred firing times of MCs relative to their local LFP (lLFP) varied with MC location in the OB. At different locations, the MC-lLFP phase differences were equal and opposite to the phase differences of LFPs. Consequently, the preferred firing times of MCs were synchronous across the bulb. The mechanisms underlying synchronous spiking yet phase-shifted LFP oscillations remain unknown.

The structures of odor-evoked activity patterns made up of synchronized (across the OB) or non-synchronized spikes were analyzed from responses of 58 MCs to 16 amino acid odorants, hypothesizing that targets of MCs process these signals differently. Each spike was classified as being synchronized or non-synchronized based on its relationship to a simultaneously recorded LFP. Synchronized spikes accounted for ~10% of all spikes and gave rise to sparse activity patterns.

Cluster analysis revealed that activity patterns of non-synchronized spikes were decorrelated over the first few hundred milliseconds of the odor response, thereby improving discriminability by observers [1]. Activity patterns of synchronized spikes, in contrast, clustered during this period: the patterns evoked by structurally related odorants became very similar, thus promoting odor categorization.

In conclusion, the preferred spike times of a distributed subset of MCs are synchronized, although oscillatory LFP activity is phase-shifted across the OB. The signals carried by patterns of synchronized and non-synchronized spikes carry different information about the stimuli when analyzed as large population vectors. They could thus subserve different functions (such as categorization and discrimination, respectively). Hence, if decoding mechanisms exist to discriminate synchronous from asynchronous spikes, various features about an odor could be multiplexed across a population of neurons.

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409 Functional organization of input to the mouse olfactory bulb glomerulus visualized with 2-photon calcium imaging

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The glomerulus receives sensory input from olfactory receptor neurons (ORNs) expressing the same odorant receptor and is thought to act as a functional unit in olfactory processing. However, whether the glomerulus is functionally homogeneous has been difficult to address. We used two-photon imaging, in combination with selective loading of ORNs with calcium-sensitive dye, to image odorant-evoked receptor neuron input within single glomeruli at high spatial and temporal resolution. Evoked calcium signals were distributed heterogeneously, with 'hot spots' of large-amplitude signal presumably representing densely packed nerve terminals. However, the hot spots appeared to be distributed with equal probability throughout the glomerular volume. Thus, we did not find any evidence for functional subdivisions within the glomerulus, at least at the level of receptor neuron input. Structurally distinct odorants presented at similar effective concentrations elicited similar spatial patterns of calcium signal within the same glomerulus. While the time course of the odorant-evoked signal was concentration-dependent, at a given concentration it was similar throughout the glomerulus, except for occasional small differences in response latency. Thus, with few exceptions, receptor neuron input to a glomerulus appears to be spatially heterogeneous but functionally uniform. These results support the hypothesis that the glomerulus acts as a functional unit and integrates sensory input from idiotypic ORNs.

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410 Cre-loxP-mediated conditional gene expression in pain pathways

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Plasticity of primary sensory neurons and of the synapses they make with dorsal horn neurons is an important component of the cellular basis of chronic, pathogenic pain. Mice genetically modified to lack particular genes and gene products are currently contributing largely to our understanding of the molecular basis of aberrent pain processing in the context of an intact organism. Owing to the multiple limitations and liabilities of global ablation, systems which enable a tightly-regulated spatial and temporal control of gene expression are required. The Cre-loxP recombinase system is the most prominent binary systems available for inducible, tissue-specific gene expression and has been

successfully used for conditional expression of a multitude of genes, including several neural genes. Cell-specific ablation of modified (floxed) gene alleles is brought about by transgenic expression of the enzyme Cre recombinase in a cell-specific manner. The bottleneck for taking full advantage of this powerful technology in pain research is the availability of mouse lines expressing Cre recombinase selectively in anatomically and functionally distinct components of pain pathways. As a prerequisite for knocking out the expression and function of nociception-relevant molecules selectively in nociceptors, we are working towards establishing transgenic mice expressing Cre recombinase selectively in nociception-specific, small-diameter neurons (C-fibers) and medium-diameter neurons (A-δ fibers) of the dorsal root ganglia (DRG). In order to generate appropriate transgenic constructs, a bacterial artificial chromosome (BAC) containing 150kb-large genomic sequences of the mouse locus of a sensory neuron-specific sodium channel, Na(v)1.8 was identified and characterized. The coding region of Cre recombinase was introduced in to the ATG site of the Na(v)1.8 coding region in the BAC via homologous recombination using the ET-cloning system. Transgenic mice derived by pronucleus injections of the correctly-recombined BAC were analysed for the level and pattern of expression of Cre protein in the DRGs, spinal cord and brain by immunohistochemistry using Cre-specific monoclonal antibodies. To verify recombinase activity of the expressed Cre protein, transgenics were mated with the Cre reporter mouse line, Rosa-26-lacZ, which express the histologically-detectable ß-galactosidase protein at high levels specifically in tissues where Cre recombinase is active. Histological analysis of co-transgenic progeny revealed Cre-mediated recombination in almost all DRG neurons of small- or medium-diameter, whereas a majority of large-diameter DRG neurons (non-nociceptive) failed to express Cre recombinase. These mice thus provide a wide scope for studying functions of a large number of interesting protein which are important in the development and function of nociceptive pathways.

Binary mixture interactions in odor-evoked patterns of afferent glomerular activity of zebrafish

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The perceived quality of an odor mixture is often distinct from the quality of its components, suggesting that the representation of the mixture is not a simple combination of the components' representations. Mixture interactions include non-linear processing such as suppression or synergism and may occur at various levels of odor processing [1-4]. Here we investigated mixture interactions at the first synaptic relay of the olfactory pathway of zebrafish. Patterns of afferent glomerular activity in the olfactory bulb (OB) were measured optically after loading of olfactory receptor neuron (ORN) axons with a calcium indicator [5].

Odor-evoked calcium signals in the OB were predominantly positive and increased monotonically with concentation. Under these circumstances it is possible to predict an activity pattern evoked by a mixture based on the patterns evoked by the components, assuming that the responses to the components do not interact. This prediction requires some knowledge about the concentration-response function, which was obtained from

measurements. Mixture interactions were then evaluated by comparing the measured response to the mixture against the predicted response, as well as against conservative limits.

The stimuli used were single amino acids or complex food extracts. Only binary mixtures were tested. With both stimulus types, mixture suppression and synergism were observed. These effects, however, were small. Glomeruli that responded to the mixture always responded also to at least one of the components; mixture interactions affected only the response intensity. Mixture-evoked patterns of glomerular input activity were therefore largely predictable from the responses to the individual components.

These results indicate that mixture interactions in the peripheral olfactory sytem are weak, for the range of stimuli and glomeruli studied here. In mitral cells, in contrast, significant interactions are observed (see abstract by Tabor and Friedrich). These mixture interactions affect both the response intensity and time course. Hence, in the zebrafish, non-linear processing of odor-evoked activity patterns is weak in the peripheral olfactory system but is performed by the nerual circuitry in the OB.

Supported by the Max-Planck-Society.

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412 Lamination of Odorant Receptor Expression Along the Basal/Apical Axis of the Zebrafish Olfactory Epithelium

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The primary event in olfaction is the interaction of odorants with G-protein coupled odorant receptor proteins. Individual olfactory sensory neurons (OSNs) most likely express only one out of a highly diverse family of receptor genes. OSNs of the same receptor phenotype are sparsely scattered throughout the sensory surface. Previous studies indicate however, that the epithelial area density of equally specified OSNs is highly inhomogeneous, amounting to four stereotyped, overlapping expression domains in zebrafish[1]. In higher vertebrates a corresponding subdivision of the main olfactory epithelium into four largely non-overlapping expression zones in its tangential dimension has been found. In contrast, the rodent accessory olfactory epithelium (vomeronasal organ) has been shown to exhibit a laminar segregation of receptor expression along its basal/apical axis[2]. We therefore decided to analyse the expression pattern of zebrafish odorant receptors in the dimension orthogonal to the epithelial surface.

To this end we performed a quantitative *in-situ*-hybridisation study on horizontal cryosections of the olfactory rosette of zebrafish, *Danio rerio*, using digoxigenylated antisense RNA probes for eight different odorant receptor transcripts. The positions of individual labelled cell bodies were determined relative to the local thickness of the sensory epithelium and analysed statistically for their distribution profile. The results

suggest a lamination of the epithelium according to receptor specificity. Distribution profiles along the basal/apical axis are markedly inhomogeneous, stereotyped and significantly different for different receptors. At least three different strata can be distinguished with multiple receptors sharing the same stratum. The laminar expression pattern of one of the receptors analysed is significantly sexually dimorphic. The organization of the zebrafish olfactory epithelium along its basal/apical axis thus resembles that of the rodent vomeronasal organ.

The function of spatial segregation of odorant receptor expression remains unknown. Conceivably grouping of receptors according to their expression profiles indicates a coregulation of the corresponding receptor genes and presumably also a coregulation with accompanying cell surface molecules causing OSN colocalisation. It might therefore provide a means of cell sorting for developmental processes like neuronal differentiation or axon pathfinding to target domains of the olfactory bulb.

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Modulation of presynaptic Ca^{2+} accumulation in insect antennal lobe projection neurons at the calyces of the mushroom body

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Whole-cell patch-clamp recordings from identified projection (output) neurons in the antennal lobe of the male sphinx moth *Manduca sexta* have shown that serotonin (5HT) increases both the excitability and their responses to stimulation with pheromone. This increase in excitability is caused by a modulation of at least two K^+ currents (J Neurosci 19(19):8172-8181). Ca^{2+} plays a critical role both in synaptic transmission and in the control of the intrinsic firing properties of neurons. Consequently, Ca^{2+} levels are a major target of neuromodulators. However, modulation of Ca^{2+} channels can be difficult to detect by voltage clamp of the soma, if Ca^{2+} channels are selectively modulated at distal regions of the neuron. Here, a combination of laser scanning microscopy and voltage clamp is used to evaluate the modulation of presynaptic voltage dependent Ca^{2+} accumulation by 5HT in synapses between antennal lobe projection neurons and Kenyon cells of the mushroom body. Ca^{2+} accumulation was stimulated by controlled voltage steps in voltage clamp. To maximize spatio-temporal resolution line scan mode with a frequency of 500 Hz was used. In distal arbors of projection neurons in the calyces of the mushroom bodies Ca^{2+} originated mostly from small spatially restricted bouton-like varicosities. In these varicoities the voltage evoked Ca^{2+} accumulation was reversibly increased by exogenous 5HT. Serotonin increased the peak amplitude, but had no effect on the rise- and decay-time. Serotonin might modify chemical synaptic transmission from projection neurons to Kenyon cells by increasing Ca^{2+} entry at neurotransmitter release sites.

414 Neuronal population responses to single odorant compounds and their binary mixtures in the antennal lobe of the cockroach, *Periplaneta americana*

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Multi-electrode neuronal population recordings where performed *in vivo*, from the first olfactory relay (the antennal lobe) of the cockroach *Periplaneta americana*, using a 16 channel data aquisition controller (IDAC2000, Syntech, Hilversum, NL) and tetrodes provided by the Center for Neural Communication Technology (Ann Arbor, MI). Spontaneous as well as odor evoked activity from projection and local neurons was recorded. Stable, long-lasting (5-10 hours) recordings were obtained. Spike-sorting was performed with the method of Pouzat, Mazor and Laurent (2002, J Neurosci Methods, 122, 43) as well as with a new method (Pouzat, Delescluse and Diebolt, see posters in *Methods and Demonstrations*). A characterization of the spontaneous activity of projection and local neurons will be presented first with a special emphasis on correlations between neurons. Responses to single odorant compounds will then be shown and the relation between response similarity between neurons and correlations in the spontaneous regime (for the same neurons) will be investigated. Responses to binary mixtures (e.g., octanol + citral) will be presented. A characterization of the relation between responses to single odorants and binary mixtures of the same molecules will be attempted. Finally a comparison with two other insect species, locust (*Schistocerca americana*) and honeybee (*Apis mellifera*) will be made.

415 Dynamics of slow components regulating spiky local field potential waves of the slug (*Limax*) brain: Application of wavelet tools

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The olfactory center (procerebrum) of the *Limax* brain is known to spontaneously discharge spiky local field potential waves. The Fourier-based method (Schütt et al., 2002b) has revealed that they are of multi-frequency components and regulated by various slow fluctuations, such as ~0.1 Hz, ~0.6 Hz, ~1.2 Hz, ~2.2 Hz and ~5 Hz and infraslow amplitude modulation. However, application of ethanol as odor stimulus alters the activity pattern from quasi-stationary to non-stationary one with amplitude and frequency evolving with time. Fourier analysis can be applied assuming stationarity for a short epoch, but wavelet analysis is more suitable to describe such nonstationary signals since it does not require stationarity (Schütt et al. 2002a). In the present study we aimed to analyze, by the wavelet method, the *Limax* brain electrical activities. *Relative*

wavelet energy (RWE) and *normalized total wavelet entropy* (NTWS) were obtained for two regions, the neuropile and the cell layer. The results showed that in the majority of trials, entropy remains relatively high (highly disordered or complexly ordered) fluctuating moderately throughout the experiment. However, in 6 cases of the cell layer, odor (ethanol) presentation evoked vigorously time evolving response where periods of increased spike amplitude and those of prolonged spike blockade alternated. Most importantly, the RWE of the 0.1-0.2 Hz component increased its bursts in relation to the spike blocking and fluctuated vigorously. Accordingly, NTWS also pendulated between extremely low and high values. This means, the system alternated between a high entropy epoch (disordered or more complex state) and a low entropy epoch (an ordered state), but, in average, the entropy became much lower (more synchronized or frequency-tuned) than that of the prestimulus period. As another important finding of this study we emphasize "mutual exclusion" seen between the 0.1-0.4 Hz components and the others, particularly the 1.56-12.5 Hz wideband component. As shown by the mutual exclusion, the discrete ~0.1 Hz activity (not an artifact) present in the *Limax* procerebrum has a dominant influence on the transient neural state. We presume that its main functional role in regulating the neuronal activities exists in its strong interaction with other components manifested as mutual exclusion, paricularly with those of >1.5 Hz. As widely known, sensory input adds complexity to the signal and the present brain model has shown how such complexity is likely to be. Any transient state is a manifestation of interdependency among the various slow fluctuations. Wavelet entropy quantifiers can visualize interactions among various frequency components and localize temporal signal ordering (synchronization) or disordering (or more complex ordering).

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Odor-aroused state of the *Helix* brain as characterized by local field potentials: Dynamics of the procerebropedal system

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Odorant stimulation causes changes in the spectral power of local field potentials in the *Helix* central nervous structures. We hypothesize that these changes mark generalized brain states. The aims of this study were to look for general features of the dynamics existing in the procerebrum-pedal ganglion feedback system in aroused states. Arousal is defined as seen by the increase in the power spectrum of the local field potentials. Local field potentials simultaneously induced by an odor stimulant in the structures of the isolated central nervous system of the snail, *Helix pomatia*, were recorded, using two steel electrodes and analyzed by single power spectra, single Frequency-Amplitude Plots and changes of RMS-voltages of signals filtered in different frequency bands.

First, odor-stimulated arousal began with a short period of quiescence, which was followed by formation of brief odor-specific activity patterns (related to odor discrimination) in the procerebrum and the pedal ganglion where certain spectra were reproducibly and consistently shown. In the following aroused state, three types of mutual exclusion took place: the major type (Type I) was between slow activities (<2.5 Hz) of the procerebrum

(a center of olfactory signal processing) and faster activities (8-50 Hz) of the pedal ganglion (sensorimotor center). As the procerebral slow wave activities began to decay, the faster activity of the pedal ganglion started to increase and continued even after removal of odor. The situation reversed as arousal diminished. This suggests that increase in the pedal functional activities simultaneously inhibits the procerebral function. Mutual exclusion between the slow and faster components was also observed intraganglionically in the pedal ganglion. In two other lesser types (Type II and Type III), exclusion (decrease in the procerebrum and increase in the pedal ganglion) was seen either only in slow potentials or only in faster components.

Second, infraslow rhythms (<0.1 Hz), such as a cycle of 9 to 13 min, were also detected as amplitude fluctuations underlying in the potentials aroused in both structures and possibly interacting with each other in counter phase.

We speculate that, in intact animals, locomotion initiated by the activation of the pedal ganglion during the aroused state would cause additonal somatosensory input into the central feedback system, and that inhibition of the procerebral function may then occur.

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417 Spiky local field potential waves of the *Limax* olfactory center (procerebrum) are regulated by slow fluctuations: The effect of ethanol

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It has been hypothesized that slow waves are unrelated to the compound, spike-like discharges previously found in the *Limax* olfactory system. To test this hypothesis we analyzed the activities recorded in two regions (neuropile and cell layer) of the procerebrum of the slug, *Limax marginatus*, applying the Fourier-based method. The central ganglia were isolated together with the tentacles and ethanol was applied as odor stimulus.

Spectral analysis [Schütt et al. 2002] showed wideband activities (up to ~25 Hz) characterized by two strong components at ~0.1Hz and ~5 Hz and lesser ones at ~1.2 and ~2.2 Hz. A clear peak at 0.6 Hz appeared only when spikes (defined as spiky waves >50 ms wide) were discharged at this cycle. These are identifiable in all preparations and there are good reasons to consider them to be brain activity. There were also amplitude modulation events - low (100 s) followed by high amplitude (100 s). On application of ethanol, both frequency and amplitude began to dynamically evolve with time, and simultaneously slower amplitude modulation events – low (200 s) followed by high (200 s) - started to dominate the activity. Notably, after removal of stimulus, spike oscillation immediately became stationary. In half the cases of cell layer recordings, however, application of ethanol led to long cessation of spikes whereas the ~0.1 Hz activity increased. A mutual exclusion seemed to exist between this slow wave and higher fre-

quency components (>1.5 Hz). It is important that this ~0.1 Hz activity with its low amplitude (1 to 2 µV RMS) has a power as high as that (4 to 5 µV RMS) of the ~5 Hz activity, since such fluctuations (~0.1 Hz) probably bias, selectively, the likelihood of neurons firing, according to their orientation, tuning, and "resting" excitability level [Bullock, 1997].

As the present results show, the *Limax* procerebral activities with the sense organs attached are more complex than has been believed. On the contrary, the procerebrum detached from the sense organ discharges a single rhythm. Any transient state observed is a manifestation of interdependency among the various slow fluctuations described above. The present method has allowed, for the first time, to evidence that slow waves seen as Fourier components and infraslow amplitude modulation events regulate the Limax brain functional activity.

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Dominance-dependent sex-pheromone response in the shore crab

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Utilisation of chemical cues during mating is a well-known phenomenon many animal groups. Sexual pheromones have been well studied in especially insects and mammals. They have also been found in crustaceans but details are less well-known in this group. In this investigation we establish the possibility that male status can affect sex-pheromone response in crustaceans.

The presence of a female sexual pheromone in the shore crab *Carcinus maenas* has been known since 1970s. In this species, mating occurs in localized hot spots, where large males compete for access to females. Around this area smaller males gather to intercept the females, who are migrating into the centre of the hot spot. The intercepting male then holds the female, and tries to keep out of reach for the larger males. This system with dominance and satellite males, may explain why some males respond differently to the same pheromone.

Using a controlled flow olfactometer, activities of subordinate or dominant conditioned male crabs, exposed to female pheromone-containing urine, were measured, and behaviours were registered.

When exposed to the sex-pheromone, subordinate large males were found to be more active than dominant ones. In small males, activity patterns were the opposite, i.e. dominant ones were more active than subordinate ones. To control if the behavioural patterns could be reversed after conditioning, large males were conditioned to be either dominant or subordinate and later reconditioned to be the opposite. The olfactometer tests here showed that when conditioned to be subordinate, the same crabs were active longer and searched about twice as often, as when conditioned to be dominant. This pattern was the same irrespective of which order the crabs were conditioned in.

Our studies support the notion that male status indeed affects his response to the female sex-pheromone, and we suggest this may be because males use different mating strategies depending on status.

419 Microdiversity of Grp1 and Grp2 Pheromone Binding Proteins in Insects: Structural Properties and Specific Function

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In insects, olfaction occurs first in the antennae which bear the sensory neurons devoted to the pheromone reception. The sensory neurons are not directly connected to the external environment, but they are surrounded by an aqueous lymph that creates a natural barrier at the air-neuron interface. This lymph is highly proteinaceous; some very specific lymphatic proteins are believed to bind to hydrophobic pheromone molecules in order to allow their solubilization and transport to the sensory receptor neurons. These proteins are called Pheromone Binding Proteins (PBPs; Vogt and Riddiford, 1981). Proteins with similar function, i.e. solubilizing and carrying pheromone/odorant molecules, have been found in the olfactory mucus of cows, therefore PBP-like molecules are not specific to insects but seem to retain important functions across the diverse animal species of the terrestrial kingdom (Pelosi et al. , 1982).

Our research interests address insect PBPs. In moths, we have discovered two groups of PBPs, Grp1 and Grp2. The Grp-1 PBPs are proteins of 143 amino acids with a molecular weight (MW) of 16.0-16.2 kDa. The Grp-2 proteins show a conserved length of 142 amino acids, with a MW of 16.2-16.5 kDa. We have shown that the Grp1 and Grp2 PBPs are characterized by specific amino acid motifs, which are located all along the protein sequence but mainly in the C terminus. We have also shown that the Grp1 PBPs are highly conserved (about 90% identity), whereas the Grp2 PBPs are more variable (about 70% identity) across the different species of moth, suggesting that Grp1s and Grp2s are assigned to specific physiological tasks (Picimbon and Gadenne, 2002; Abraham et al. , 2002).

Interestingly, we have demonstrated the existence of a strong microdiversity in both Grp1 and Grp2 PBPs. In each group of proteins, we identified at least three different subtypes. The different subtypes of Grp-1 found in Agrotis ipsilon (Aips-1a, Aips-1b and Aips-1c) differ by the change of four amino acids, located all along the molecule. In this species the different subtypes of Grp2 (Aips-2a, Aips-2b and Aips-2c), differ by the change of ten amino acids at defined positions, mainly in the C-terminus. In a closely related species, Agrotis segetum, three subtypes of Grp1 and Grp2 have also been identified (Abraham et al. , 2002). All these observations suggest that microdiversity exists for all PBPs and most probably in most moths.

We can speculate that the amino acids that are Grp-specific or specific to a Grp-PBP subtype may have a functional importance in pheromone reception. We are currently evaluating the role of each amino acid in the attachment of pheromone molecules to the

PBPs by physical studies and directed mutagenesis performed on recombinant pheromone-PBP complexes.

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Drosophila odorant receptors in noctuid moths 420

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The indisputable discriminative chemosensory power of the insect antennae has brought moths, flies, mosquitoes and locuts to the top of the emblematic models for studying chemosensors and more particularly the principles of odour perception. As a first step in this process, chemical signals are transduced in electrical signals by G-protein coupled receptors (GPCRs) known as odorant receptors (ORs); the molecular interaction of an odorant with the chemosensitive dendritic membrane of OR neurons initiates a series of membrane events leading to sensory transduction and impulse initiation. Olfactory signal transduction is remarkably consistent across living organisms. In nematodes and mammals, our knowledge has been greatly clarified by the discovery of a multigene family encoding a large array of ORs. In contrast, insect ORs have remained an open field due to the failure in cloning putative ORs from the antennae. From genomic databe analysis, a novel family of putative ORs in the fly *Drosophila melanogaster*(DORs), the mosquito Anophaeles gambiae (AgORs) and the moth Heliothis virescens (HRs) has been described. These molecules are members of a highly divergent family of receptors, displaying between 10% and 75% identity and bearing no sequence homology to any other GPCRs. They may represent insect-specific molecules, but what about the existence of DORs in other organisms?

In order to address the question of odorant-receptor interaction properly, in particular the functional expression of DOR proteins, we have cloned DOR orthologous genes in various noctuid species that use different pheromone systems and discussed the role of DORs in pheromone reception.

Molecular Evolution of Odorant-Binding Protein Genes in Moths 421

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Reception of odorant molecules is crucial in insects since they largely describe location and quality of suitable food sources, host-plants and mating partners. The insect antennae erected on the head in direct contact with the environmental air are responsible of the capture of the odorants. The odorants are “caught” by thousands of olfactory hairs

(or sensilla) that contain the perceptive structures, the olfactory receptors of the neuronal dendrites of sensory cells. Like the mucus from vertebrates, the sensillar lymph bathes the olfactory dendrites and prevents the odorant molecules (hydrophobic in nature) to contact directly the dendritic receptors. A solubilization process is required. A class of small soluble proteins, the odorant binding proteins may allow the solubilization of the odorant molecules in the sensillar lymph and may facilitate their interaction with the sensory receptors (OBPs; Vogt and Riddiford, 1981).

In Lepidoptera, the OBPs are traditionnaly regarded as pheromone or general odorant binding proteins (PBPs and GOBPs; Vogt et al., 1991). Both PBP and GOBP proteins display a strong heterogeneity and different groups of proteins have been documented (PBP1/PBP2, GOBP1/GOBP2; Picimbon and Gadenne, 2002; Vogt et al., 1991). The PBPs are mainly expressed in pheromone-sensitive sensillae from the male antennae. In contrast, the GOBPs are preferentially found in plant odorant-sensitive sensillae from the female antennae (Vogt et al., 1991; Steinbrecht et al., 1995). This suggests that PBPs and GOBPs may have evolved separately over the course of plant evolution and the development of different pheromone systems.

To explore Evolution in OBPs, we identified the genes encoding PBPs and GOBPs in different Lepidopteran species. Our study reveals that GOBP and PBP genes all have the same 3 introns – 2 exons structure but markedly differ by the intron length (Abraham et al. , 2002; Picimbon, 2003). Similar variations are observed within the genes encoding different PBP subtypes, suggesting that in OBP genes the introns contain potential sequences for gene regulation and expression of specific OBPs.

Evolution may differently affect the olfactory genes with respect to the specific functions of OBP and to the specific variations of the external odorant environment.

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Chemosensory protein diversity among the insect orders as indicated by a CSP-related protein of the flour beetle *Tribolium freemani* (Coleoptera)

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The Tribolium flour beetles are associated with stored food since more than 4000 years. They remain beyond the most important stored product pests. They have first evolved as saprophylic insects and naturally occur under the bark of trees or in dead wood, mould and the nest of some Hymenopteran species. The adaptation of Tribolium to breeding in the environment of dry stored products has been observed in several species of this genus, as in most other insect species occurring in this environment. The possible adaptation of chemical communication in insects for their coexistence in the same habitat is virtually unexplored. Beetles of more than one Tribolium species may occur in sympatry in the same environment and inter-specific attraction was observed between species, suggesting imperfect pre-reproductive isolation between some species. Moreover, sterile hybrids are observed between T. castaneum and T. freemani and between T. audax and T. madens. Flour beetles are therefore good model species to examine the evolution of insect chemical communication as a secondary adaptation to allow breeding in a common environment.

Insects monitor their chemical environment through specialized sensilla, present in different parts of their body (antennae, mouth organs, legs, "…"). Within olfactory sensilla, small soluble proteins in the microenvironment of sensory receptors have a crucial function in chemical detection. The best studied are odorant binding proteins (OBPs) that have been discovered in a high diversity of insect species and orders. A new class of proteins, the chemosensory proteins (CSPs), expressed in general chemosensory organs such as legs, has recently gained increasing interest after their discovery in many different insect species, not only in the hemimetabolous Orthoptera but also in the holometabolous Diptera, Lepidoptera and Hymenoptera.

Here, this is the first report on CSP from Coleopteran species. The protein CSPs from Tribolium freemani show: (a) a close sequence similarity (around 70-80%) with CSPs from Lepidoptera such as the noctuid Mamestra brassicae and Heliothis virescens, (b) the presence of a signal peptides and cysteine motifs (four conserved cysteins), (c) tissue-specificity and (d) interspecific variability.

423 The TRPV2 channel (VRL-1) is constitutively expressed in the primary sensory cell line F-11: Molecular and functional characterization

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Members of the vanilloid receptor family (TRPV) are activated by a diverse range of stimuli, including temperature, protons, phorbols, lipids, changes in extracellular osmolarity and pressure, and depletion of intracellular Ca^{2+} stores. The temperature-sensitive TRPV channels (TRPV1, TRPV2, TRPV3, TRPV4) also differ in their thermal response thresholds and their cellular distribution pattern in primary sensory neurons.

Here we characterize the heat-sensitive vanilloid receptor-like TRPV2 channel (also known as VRL-1), which is endogenously expressed in the neuronal cell line F-11 (hybridoma of mouse neuroblastoma and rat dorsal root ganglion cells). The isolated 2,7kb full length cDNA from F-11 cells was 100% identical to the mouse TRPV2 channel. Using RT-PCR and Northern blot we described a single TRPV2 transcript in the F-11 cell line and the lack of the capsaicin-sensitive TRPV1 channel (VR1). The proper RNA translation into TRPV2 protein was confirmed by immunocytochemistry. TRPV2 immunoreactivity was detected in the cytoplasm and at the cell membrane as assessed by confocal laser microscopy.

Electrophysiological studies using the whole cell patch clamp technique on isolated F-11 and mouse VRL-1 transfected HEK293 cells demonstrated a heat-evoked increase in outward and inward current with a threshold of 51.6 ± 0.2°C (n = 15), which could be reversed at 37°C. Current-voltage relation of 52°C stimulated F-11 cells (Cm = 25.9 ± 2.3 pF, n = 16) showed an outward rectification with a reverse potential close to –10mV. Current density amplification of 5.3 ± 1.5 -fold at –60 mV and 5.1 ± 0.8 -fold at +60 mV was observed after the same stimulation (n =15). There was no activation of F-11 and transiently transfected HEK293 cells by stimulation with capsaicin (n = 6).

Our results suggest that functional TRPV2 channels are constitutively expressed in F-11 cells and, therefore, that these primary sensory cells are most likely derived from medium-sized noxious heat transducing Ad afferents. Thus, F-11 cells may serve as a valuable *in vitro* model to study the transcriptional regulation of the TRPV2 gene and to elucidate the TRPV2 mediated intracellular signalling cascades in sensory neurons in response to noxious heat.

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Effects of cyclic nucleotides on cultured olfactory receptor neurons and on olfactory sensilla of the hawkmoth *Manduca sexta*

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Strong and prolonged pheromone stimulation causes rises in intracellular cGMP concentrations which correlate with time courses of olfactory adaptation (Ziegelberger et al. 1990, J Neurosci 10:1217). We hypothesize that this state of adaptation is reversed by octopamine-dependent elevation of cAMP concentrations. Thus, in extracellular tip recordings of antennal trichoid sensilla of male *Manduca sexta* moths, we investigated the effects of cyclic nucleotides on pheromone transduction. When a bombykal (BAL) stimulus of 10 µg dose and 50 ms duration was applied every 5 min, the sensillar potential and action potential responses remained constant during 3 h of repetitive stimulation. After inclusion of 10 mM 8-bromo cGMP in the tip recording electrode, the action potential frequency was continuously reduced. In addition, the latency of the first action potential was prolonged, while the amplitude of the sensillar potential was not altered. Furthermore, the stimulus-correlated reduction of the action potential amplitude was less pronounced in the presence of 8-bromo cGMP. These effects indicate an 8bcGMP-dependent conductance decrease.

Preliminary results suggest that also 8-bromo cAMP (8bcAMP) modulates pheromone transduction. During perfusion of the sensillar lumen of BAL-stimulated olfactory sensilla with 10 mM 8bcAMP the sensillar potential increased continuously over a period of 3h, possibly due to a resistance increase, while the action potential response remained unchanged at a high frequency. Perforated patch clamp recordings of cultured ORNs distinguished 11 types of currents by their current-voltage relations. Application of 8bcAMP decreased the cation currents I_{LL} and $I_{cat?}$ as well as the potassium currents $I_{K(cGMP)}$ and I_{Ki}. While $I_{cat?}$ shares properties with a protein kinase C-, TEA-dependent, non-specific cation current, I_{LL} is a long-lasting cation current, which activates with depolarization and inactivates slowly with hyperpolarization. We hypothesize that I_{LL} and $I_{cat?}$ are activated only via long-lasting rises of intracellular Ca^{2+} during adapting pheromone stimulation, but are not activated by non-adapting pheromone stimuli. $I_{K(cGMP)}$ is a cGMP-, cAMP-, and ATP-blockable delayed rectifier, non-inactivating K^+ current, while I_{Ki} is a slowly inactivating delayed rectifier type K^+ current. Both K^+ currents are activated at prolonged depolarizations as occur during prolonged or strong pheromone stimulation. Our hypothesis is that circadian rises in octopamine before the beginning of the night disadapt olfactory sensilla via cAMP-dependent mechanisms to allow for better olfactory discrimination and temporal resolution during the activity phase of the moths. [Supported by DFG-grants STE 531/10-1,2,3]

425 Receptor sensitivity underlies the behavioural effectiveness of chemosensory avoidance movements of the legs of locusts.

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Unlike vertebrates the taste receptors of insects are distributed over the mouthparts, body and legs. In insects the sense of taste is used to select and reject food, select appropriate egg-laying sites and to avoid noxious chemicals in the environment. Here we have analysed some of the coding properties of the taste receptors, or basiconic sensilla, on different regions of the legs of locusts and related them to behavioural responses evoked through chemical contact.

We analysed the responses of taste receptors to two behaviourally relevant chemicals, sucrose and sodium chloride (NaCl). In response to stimulation with 500mM NaCl sensory neurones innervating receptors along the dorsal or ventral surfaces of the hind and fore legs progressively produced more spikes towards the tarsus, so that tarsal taste receptors responded at twice the frequency (approx. 20Hz) of proximal sensilla (approx. 10Hz). Similarly, 100mM sucrose applied to distal receptors on the hind and fore legs evoked more spikes than when applied to proximal receptors. Basiconic sensilla tested with a range of NaCl concentrations were more sensitive on the tarsus compared to proximal sites.

Behavioural studies showed that the frequency of leg withdrawal movements to droplets of chemicals applied to a leg increased with increasing NaCl and sucrose concentrations. For a given concentration the frequency of withdrawal was greater when it was applied to the tarsus compared to proximal femur. The foreleg was more responsive than the hind leg to NaCl whereas the frequency of withdrawal was similar to sucrose for both legs.

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426 An olfactory receptor expressed in ganglia of the autonomic nervous system

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Recent studies of genetically manipulated mice have provided evidence that olfactory receptors (ORs) may have important functions in addition to recognizing odorous compounds; receptor proteins apparently play a role in targeting olfactory axons to their correct glomerulus in the olfactory bulb. It has been suggested that they may contribute to the complex process of precise cell-cell recognition. These observations have shed some new light on the originally obscure finding that some of the OR genes are expressed not only in olfactory sensory neurons (OSNs) but also in other tissues. The results have vitalized the idea that OR proteins participate in cell recognition and tissue formation in these organs, culminating in the so-called area-code hypothesis. In this

concept it has been proposed that the precision of cell migration and tissue assembly during embryogenesis requires a complex addressing system. Due to their enormous diversity and proven role as address molecules in the olfactory system, ORs have been considered as possible candidates serving this function. We have identified a novel OR gene (MOL2.3) which was found to be expressed not only in OSNs but also in subpopulations of neurons in distinct brain regions. In this study we set out to examine whether this OR subtype is expressed in tissues other than the nasal epithelium and brain, and tried to identify the target cells for MOL2.3 expressing cells. For this purpose, the transgenic mouse line MOL2.3-IGITL has been analyzed in which Green Fluorescent Protein (GFP) and the axonal marker τ-ß-galactosidase are expressed under control of the MOL2.3 promotor. The olfactory receptor subtype MOL2.3 was found to be expressed in distinct subpopulations of cells within a cranial, a cervical as well as within a thoracic ganglion. The axonal processes of MOL2.3 expressing cells could be visualized and thus the target tissues innervated by these ganglionic neurons identified. Stained fibers, but no stained cell bodies were visible in distinct head regions, notably in the lateral nasal gland and in the so-called Harderian gland; staining was also observed on segments of blood vessels, especially within the tongue. In the thoracic region, the heart and a small segment of the aorta as well as a distinct population of lung alveolae were labeled by incoming fibers. Expression of MOL2.3 in cells of the autonomic nervous system supports the idea that at least some of the multiple olfactory receptor types serve functions others than odorant detection.

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Olfactory receptors in the mouse septal organ 427

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Mammalians have three different chemosensory subsystems: the main olfactory epithelium (MOE) spanning cartilaginous lamellae called turbinates, the vomeronasal organ (VNO) a tubular structure enclosed in the septal vomer bone and a small epithelial patch on the nasal septum at the entrance to the nasopharynx called the septal organ (SO, organ of masera). While the MOE is considered to be responsible for the reception of general odorants and the VNO is thought to mediate the detection of substances carrying specific information concerning species, gender and identity of an animal, almost nothing is known about the molecular organisation and functional role of the SO.

Therefore we set out to identify the receptortypes which are expressed in sensory neurons of the SO. Transgenic OMP-GFP mice, exhibiting green fluorescence within mature olfactory neurons allowed us not only an easy identification, but also a contamination-free isolation of the SO. PCR analyses of cDNA with degenerate primers matching conserved motifs of olfactory receptor (OR) genes were then carried out: 50 different OR types were identified. These receptors could be attributed to 27 families and all belonged to receptor class II (mammalian specific), none of the class I OR types (fishlike) was found in the SO. *In situ* hybridization analyses revealed that particular OR

types were expressed in very different numbers of cells; they could be categorized into 3 groups: group-1 receptors were found to be expressed in less than 100 out of about 3300 mature olfactory neurons; group-2 receptors were found in 300-400 cells and group-3 (one receptor type) was found in around 1000 cells.

As the SO develops from the ventral part of the perspective MOE it was considered that only receptors of the ventral zone may be expressed in the SO. Therefore all SO receptors were assessed for expression within the MOE. It was found that out of 47 definable patterns 12 OR types were expressed in the ventral zone, 33 in the medial and only 1 OR type was expressed in the dorsal zone. Analyses for expression patterns in the SO revealed an inhomogenous distribution of OR expressing cells, but no indication for a zonal organisation was observed. As an approach to elucidate the projection pattern of the SO neurons anterograde DiI-tracing experiments were performed. The studies led to a staining of a very distinct set of glomeruli in the ventromedial region of the olfactory bulb. Labeled fibers were observed in 318 glomeruli located in a defined posterior area. Three types of glomeruli could be discriminated: glomeruli containing single fibers (247), glomeruli partially stained by DiI labeled fibers (66) and glomeruli completely stained by DiI labeled fibers (5). Taken together this study provides the basis to understanding the chemosensory role of the septal organ.

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428 OR37-receptors: A unique subfamily of olfactory receptors

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While sensory neurons expressing distinct olfactory receptor types are usually distributed within broad rostro-caudal zones, those neurons expressing an OR37 receptor are assembled in a small area of the nasal neuroepithelium in a variety of mammalian species. A comparative analysis of the mouse and human genome revealed that in both species OR37 genes are organized in two clusters: cluster-I comprising 5 genes and cluster-II containing 3 genes in mouse and 7 genes in human. The pronounced diversity of non-coding sequences in both genomic loci indicates a long coexistence of the two gene clusters and the genes within the clusters, nevertheless the coding regions showed a remarkably high level of sequence identity; thus indicating that OR37 genes may be under negative selection pressure in contrast to normal olfactory receptor genes and suggesting that the OR37 receptors may be tuned to recognize distinct sets of signaling molecules. Towards an understanding of the mechanisms controlling the distinct OR37 expression pattern, the genomic sequences upstream of the receptor coding regions from the two loci were studied in detail. 5`-RACE experiments and bioinformatic analyses revealed complex intron/exon structures and led to the identification of short DNA stretches of approximately 150 bp immediately upstream of the transcription start sites common to all genes of these clusters. Comparative studies on the rat and human OR37 repertoire demonstrated the existence of similar sequence motifs also across species border. To test whether these sequence motifs may be part of the expression control machinery for OR37 genes, a representative mOR37 5`sequence was employed in gel-shift assays, demonstrating specific binding only of proteins from olfactory epithelium

nuclear extracts. Furthermore, yeast one-hybrid experiments demonstrate that distinct transcription factors expressed in the olfactory epithelium interact with this region. These results suggest that the DNA-motifs present in the 5`-non-coding regions of the OR37 genes may be important for the regulation of these genes.

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Subzonal organization of olfactory sensory neurons projecting to distinct glomeruli 429

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Olfactory information is conveyed from the nasal neuroepithelium to the main olfactory bulb (MOB), the first relay station in the brain, via the axons of olfactory sensory neurons (OSNs). Each neuron extends a single axon to the bulb where it synapses onto the dendrites of mitral or tufted cells in a characteristic spherical region of neuropil, called glomerulus. Neurons expressing a particular odorant receptor gene are located within a defined zone of the epithelium and selectively project to a distinct domain of the MOB. Transgenic approaches have demonstrated that all the axons of neurons expressing the same receptor converge onto common glomeruli in the bulb. Surprisingly, they do not target a single, but in most cases two, often distantly located glomeruli, one in the medial and one in the lateral hemisphere of the bulb. These results suggest that the assembly of OSNs expressing a given OR-type can be divided into two subpopulations: one group directing their axons to a medial glomerulus and one group projecting to a lateral glomerulus.

In the present study we have analyzed transgenic mouse lines in which the projection of a receptor-specific neuron population can be visualized due to axonal markers which are co-expressed. The target glomeruli could thus reproducibly be identified and allowed to precisely deposit retrograde tracers. After an appropriate incubation time OSNs within distinct areas of the olfactory epithelium were labeled. Applying different fluorescent dyes to the medial and lateral glomerulus, respectively, two populations of OSNs were stained that are segregated within the expression zone. This characteristic segregation of neurons into two spatially separated populations was observed for neuron populations from different expression zones.

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Evolution of the OR37 subfamily of olfactory receptors: A cross-species comparison 430

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OR37 receptors represent a subfamily of olfactory receptors which have several special properties, including an extended third extracellular (EC3) loop and a clustered expres-

sion pattern in the olfactory epithelium. They were first discovered by a PCR-based approach in rodents. The recent completion of large-scale sequencing of the mouse genome has allowed the characterisation of the complete repertoire of the OR37 receptor subfamily of the mouse. A total of 8 OR37 genes exist in mouse. Uniquely among olfactory receptor subfamilies, they are organised in 2 clusters. Cluster-I consists of 5 genes, which show a very high level of sequence homology to each other (approx. 90%). Cluster-II consists of 3 genes, with less sequence similarity than within the 1st cluster (approx. 60%). The ongoing sequencing of the rat genome allows us to investigate the genomic organization of the rat OR37 receptor genes. Searching the rat genome database we found 1st and 2nd clusters, containing 5 and 9 genes, respectively. As is observed in the mouse, the genes of rat cluster-I show very high sequence similarity to each other. In contrast to the low similarity mouse cluster-II genes, 7 genes of rat cluster-II have high sequence similarity to each other leaving two with lower similarity.

In order to get more detailed insight into the evolution of this particular OR gene subfamily, the inspection of orthologous genes in other species could be informative. The detailed sequence information of the human genome was therefore utilised. As in rodents, there are 2 human clusters. Cluster-I, like in rodents contains 5 genes with a very high sequence similarity. Pseudogenisation has occurred with 4 of the human cluster-I genes. Cluster-II has expanded to 7 genes and this is surprising given the general trend of pseudogenisation of olfactory receptors in human.

Knowledge of the genomic organisation of OR37 receptors in a branch further down the mammalian evolutionary tree may help build a picture of how and when they evolved.

Having diverged from eutherians approximately 140-165 million years ago, marsupials represent a unique midpoint between existing mammalian and non-mammalian vertebrate models. Using a variety of degenerate primers we have cloned OR37 receptors from *Monodelphis domestica* (the laboratory opossum) genomic DNA. So far five OR37 genes were found in opossum. They have a high nucleotide sequence identity (about 70 - 80%) to those of other species. The presence of OR37 receptors in opossum supports the notion that this is an ancient family of olfactory receptors.

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431 Molecular assembly of cAMP-mediated olfactory signaling pathways via scaffolding proteins

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Odor detection via odorant receptors in the cilia of chemosensory neurons from the main olfactory epithelium induces cellular responses by stimulating adenylyl cyclase, leading to a rapid and transient elevation of the cAMP concentration which triggers the opening of cyclic nucleotide-gated ion channels. The rapid kinetics of the olfactory signaling cascade suggests that the functional elements may be organized in multimeric complexes by scaffolding proteins via their multiple docking sites for signaling proteins. A-

kinase anchoring proteins (AKAPs) function as scaffolding proteins for the assembly of signaling complexes involved in many cAMP-mediated pathways. It therefore seems conceivable that AKAPs may also be important for olfactory signaling.

Overlay assays using regulatory A-kinase subunits as probes reveal that several distinct AKAPs are present in protein samples from isolated olfactory cilia. Molecular cloning strategies, including RT-PCR-approaches and Yeast-Two-Hybrid screenings, demonstrated that several AKAP-subtypes, such as AKAP150, Yotiao, Ezrin, MAP2 and D-AKAP2 are expressed in olfactory tissue. Approaches employing specific antibodies showed immunoreactivity for Ezrin in the ciliary layer of the olfactory epithelium, moreover immunoreactive polypeptide bands of AKAP1 were visualized in Western blots of isolated ciliary fractions. Biochemical experiments monitoring the formation and hydrolysis of cAMP were performed in the presence of the AKAP-derived peptide Ht31 which disrupts interactions of AKAPs with other proteins. The results of these assays provide the first evidence that AKAPs are indeed of relevance for the cAMP-mediated olfactory signaling cascade. These findings indicate that AKAPs may contribute to the assembly and regulation of olfactory signaling complexes.

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Olfactory receptors in nonsensory neurons 432

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During critical phases of mouse development, cells expressing distinct olfactory receptors are not only found in the nasal neuroepithelium, but also in the nasal mesenchyme which separates the epithelium from the developing telencephalic vesicle. Most of these extraepithelial cells were found to be located adjacent to outgrowing axons of olfactory sensory neurons navigating from the nasal epithelium towards the olfactory bulb. Interestingly, each cell population expressing a distinct olfactory receptor type was specifically associated with axons of those neurons which express the same receptor type. Neither the origin and fate nor the function of the extraepithelial cells expressing olfactory receptors is known, however, it is conceivable that they might either be guide posts for the outgrowing axons or they might migrate along the axons into the telencephalon. Towards a molecular analysis, appropriate pieces of tissue were isolated from sections by means of the laser pressure catapulting methodology. The isolated mRNA was converted into cDNA which was subsequently assessed for novel receptor types which may be expressed in the extraepithelial tissue; thus to evaluate if all or only distinct receptor types are expressed and also if there are any spatiotemporal expression patterns. Whether extraepithelial cells may be segregated in distinct spatial patterns - in accordance with the zonal distribution of sensory neurons and the projection patterns of their axons - was analysed by mapping the location of receptor specific extraepithelial cells. Towards a more detailed characterization of the receptor expressing extraepithelial cells, a molecular phenotyping was performed, determining which expression of the characteristic marker proteins, such as Gap-43, OMP or AC-III may be expressed. These studies have led to the discovery of a unique cell population; these cells are not only unusually numerous but also exhibit a distinct molecular phenotype. Cells expressing receptors in the

nasal mesenchyme can only be observed transiently during early developmental phases; they seem to be completely vanished by embryonic day 16. Therefore, studies were performed to evaluate if these cells change the molecular phenotype or rather undergo apoptosis.

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433 Identification of novel taste-specific genes using differential screening approaches

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Identification of genes specifically expressed in taste tissue is supposed to provide new insight about the molecular mechanisms underlying recognition and transduction of gustatory signals in lingual sensory cells. Towards this goal we have performed differential screening approaches using taste tissue versus non-sensory lingual epithelium; three subtractive cDNA libraries were prepared from isolated foliate and fungiform taste buds. The isolated cDNA clones were partially sequenced and by searching the relevant databases known genes as well as redundancy were eliminated. Differential-RT-PCR experiments with cDNA from sensory and non-sensory epithelium were performed as a second step to determine those genes which are exclusively expressed in taste sensory tissue. Subsequent *in situ* hybridization studies allowed to define the topographic expression patterns on circumvallate, foliate and fungiform papillae; depending on the gene type, labelled cells were found in all or in subsets of the papillae. The sequenced full length clones were assessed by diverse bioinformatic tools towards a characterization of the encoded proteins. So far, a novel taste-specific regulator of G protein signaling termed RGS21 has been identified and several other transcripts are currently under investigation. These results indicate that differential screening approaches can lead to the identification of novel taste-specific genes.

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RGS21 is a novel regulator of G protein signaling selectively expressed in subpopulations of taste cells 434

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G protein-mediated signaling processes are involved in sweet and bitter taste transduction. In particular, the G protein α-subunit gustducin has been implicated in these processes. One of the limiting factors for the time-course of cellular responses induced by tastants is therefore the intrinsic GTPase activity of α-gustducin which determines the lifetime of the active G protein complex. In several signaling systems specific 'Regulator of G protein Signaling' (RGS) proteins accelerate the GTPase activity of G protein α-subunits. Using differential screening approaches, we have identified a novel RGS protein termed RGS21 which represents the smallest known member of this protein family. RT-PCR, immunohistochemistry and *in situ* hybridization experiments demonstrated that RGS21 is selectively expressed in taste tissue where it is found in a subpopulation of sensory cells. Furthermore, it is coexpressed in individual taste cells with bitter and sweet transduction components including α-gustducin, phospholipase Cbeta2, T1R2/T1R3 sweet taste receptors and T2R bitter taste receptors. *In vitro* binding assays demonstrate that RGS21 binds α-gustducin in a conformation-dependent manner and has the potential to interact with the same G-α subtypes as T1R receptors. These results suggest that RGS21 may play a regulatory role in bitter as well as sweet taste transduction processes.

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A candidate olfactory receptor subtype highly conserved across different insect orders 435

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In insects the detection and transduction of olfactory signals requires receptors located in the dendritic membrane of antennal sensory neurons. Genes encoding candidate insect odorant receptors were identified by analyzing the genome databases of the fruitfly *Drosophila melanogaster* and the mosquito *Anopheles gambiae*. Recently, we have characterized the first candidate olfactory receptors of the moth *Heliothis virescens* and found that they were diverse among each other and extremely divergent from receptors of the dipteran species. However, there is one exception; the moth receptor type HR2 shares a rather high degree of sequence identity (67 - 69 percent) with the olfactory receptor type Dor83b from *Drosophila* and AgGPRor7 from *Anopheles*. Moreover, this

unique receptor type was found to be expressed in numerous antennal neurons, in contrast to all other receptors. We now have identified HR2 homologues in two additional lepidopteran species, the moths *Antheraea pernyi* and *Bombyx mori*, which share 86 - 88 percent of their amino acids. In addition, RT-PCR-based experiments led to the discovery of HR2 homologues in antennal cDNA of the honey bee (*Apis mellifera*; Hymenoptera), the blowfly (*Calliphora erythrocephala*; Diptera) and the mealworm (*Tenebrio molitor*; Coleoptera). Comparison of all HR2-related receptors revealed a high degree of sequence conservation across insect orders. *In situ* hybridization of antennal sections from the bee and the blowfly support the notion that R2-type receptors are generally expressed in a very large number of antennal cells. This, together with the high degree of conservation suggests that this unique receptor subtype may fulfil a special function in chemosensory neurons of insects.

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436 A divergent family of candidate olfactory receptors in the moth *Heliothis virescens*

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For many insects the ability to detect olfactory cues from the environment is an essential prerequisite for survival and reproduction. In moths odor perception and recognition is accomplished by antennal sensory neurons housed in sensillar hair structures. Pheromones and odorants are supposed to be recognized and discriminated by seven transmembrane domain receptor proteins located in the dendritic membrane of these cells. Due to their key role in the initial step of odor recognition and their proposed potential as possible targets for novel agents to control pest insects, intensive efforts have been employed over the past decade to identify the olfactory receptors in moth, however, their molecular identity remained elusive. We have assessed a genome database of the moth *Heliothis virescens* for sequences which may encode heptahelical receptors and employed exon-specific probes to screen an antennal cDNA library of the moth. Analysis of the isolated cDNA-clones led to the discovery of a divergent gene family encoding putative seven transmembrane domain proteins. Using receptor-specific primers in RT-PCR experiments with different tissues, several subtypes were found to be specifically expressed in the antennae, supporting the notion that they may encode candidate olfactory receptors of the moth. Moreover, *in situ* hybridization experiments revealed that they are indeed expressed in antennal sensory neurons and demonstrated that each receptor subtype appears to be expressed in a distinct population of sensory cells. RT-PCR analyses of receptor expression during development indicated an onset of expression up to five days before eclosion and differences between subtypes.

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Calcium responses to queen pheromones, social pheromones and plant odours in the antennal lobe of the honey bee drone *Apis mellifera* L. 437

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The functional role of honey bee males (drones) in the colony is to mate with queens, a behaviour relying heavily on the olfactory detection of queen pheromone. How the brain of drone honey bees processes olfactory stimuli, in particular pheromones, is still largely unknown. Anatomically, olfactory receptor cells located in sensillae on the antennae project to the antennal lobes (AL), the first brain structures to process olfactory information, forming a number of functional units, the glomeruli. In drones, there are about 100 glomeruli and an additional 4 large compartments which form the macroglomerular complex (MGC). Because in moths the MGC is responsible for sexual pheromone detection and processing, such a function was also proposed in drones. One of these compartments (MG1) is particularly prominent at the surface of the AL, together with about 30 usual-size glomeruli, which makes them accessible for optical imaging studies. Using calcium imaging, odor-evoked responses were measured in the glomeruli of the drone AL. Seventeen different odors belonging to three main classes of stimuli were presented: (i) queen pheromonal components, used by drones for the recognition of queens during nuptial flights, (ii) social pheromonal components used for social cohesion in the colony, and (iii) floral odors, which are present in the food stores of the hive and/or brought back by foragers. All three classes of stimuli produced signals in a variety of glomeruli of the antennal lobe. Queen pheromonal compounds, in particular 9ODA and 9HDA induced signals in the MGC, whilst HOB and HVA each triggered activity in one, but not the same, ordinary glomerulus. Social pheromones and floral odors evoked responses in a combinatorial manner in ordinary glomeruli. This work suggests that the most active queen pheromonal components are processed in the macroglomeruli of the drone AL, and that social pheromones and floral odors, as well as other queen pheromone components are processed in ordinary glomeruli. These results are discussed in relation to the behavior and role of drones in the honey bee society, as well as with regards to the evolution of sexual signals in social insects.

Chirality and Odor Perception 438

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The concept of "chirality" is a well known phenomenon in chemistry and means - in simple terms - "handedness" and describes the existence of left/right opposition. Chirality plays an important role in many interactions with biological sites such as drug re-

sponse, insect communication or human odor perception. For humans a variety of optical enantiomers (pairs of mirror-symmetric, nonsuperimposable molecules that differ only in optical activity) have been described as having different odor qualities and/or different intensities as determined by threshold measurements. The most universally found discriminable pairs are carvone and related substances: D-carvone has a caraway note and L-carvone more of a spearmint note.

There is evidence that receptor proteins for odorants are glycosylated and several studies show that lectins (carbohydrate-binding proteins that specifically bind to lipoproteins via their sugar specific site) adhere to olfactory receptor sites. For instance, the lectin Peanut Agglutinin has been shown to block the binding of L-alanine but not L-arginine to catfish olfactory cilia. To further study this phenomenon we established olfactory thresholds for some enantiomeric odors and demonstrated that lectins (ConA, WGA) applied to the olfactory epithelium selectively block detection of only one odor of an enantiomeric pair while the response of the other odor remained unaffected. These results strongly indicate that the detection of enantiomers of at least some odors is mediated by different populations of sensory neurons.

439 TRIS-buffer decreases rat's sensitivity to odorants

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TRIS is commonly used as a buffer in histology but also in medicine to cure metabolic acidosis, as a gastrointestinal and diuretic drug as well as a solvent of drugs delivered in aerosol containers. Mainly because of the application as a solvent in aerosol containers the question arose weather TRIS affects odor detection ability. Using operant conditioning, male Wistar rats were trained in an air dilution olfactometer to respond to low level concentrations (10-3 % of vapor saturation) of odorants (ethyl acetate or n-octanal) but not to clean air. Rats mastered this task with 90-100 % correct responses. Intranasal perfusion of the olfactory mucosa with Ringer solution did not impair detection performance. However, intranasal perfusion with TRIS-buffer resulted in a reduced detection performance 30 min after treatment of the olfactory mucosa to 61.4 ± 5.6 % (ethyl acetate) and 49.3 ± 5.3 % (n-octanal) correct responses, which represent random choice levels. Increase of odor concentration to 10-2 vol % 50 min after flushing the nasal cavity with TRIS-buffer improved detection performance (95 % correct answers). Four hours after treatment, the detection performance returned to pretreatment levels (90.0 ± 12.2 % ethyl acetate; 91.0 ± 10.2 % n-octanal). We conclude that TRIS reduces (but not totally inhibits) olfactory sensitivity unspecifically and reversibly probably by changing either 1) the pH of the mucus or receptor cells and therefore the activity of enzymes in the signaling cascade or 2) by direct interaction with the odorant ligands or the odorant receptors. The underlying mechanisms have to be evaluated.

Micro- and macrosmat workers in *Bombus terrestris*: Allometry in an olfactory system and its consequences for olfactory sensitivity 440

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In contrast to all other eusocial bees, bumble-bees exhibit a pronounced intra-colonial worker size variation. Body mass differences between small and larger workers can reach up to a factor of ten.

In the last years we started to investigate the effect of body scaling on various subcompartments of the olfactory in *Bombus terrestris*. We could show that sensilla number (sensilla placodea: 708 to 2594 per antenna), sensilla density (2377 to 3168 per mm^2), volume of antennal lobe neuropil (5.7 to 19.0 $x10^6$ μm^3) and volume of single identified glomeruli correlate significantly with worker size (head width 2.7 to 4.3 mm). Cell counts indicate that these these morphological and anatomical differences are in part a result of remarkable differences in the number of sensory neurons as well as the number of antennal lobe neurons.

Using a Y-maze experimental set up, we found that differences in the olfactory system affect olfactory reaction thresholds: Larger workers have significant lower behavioural thresholds than smaller workers, indicating a higher olfactory sensitivity. Additionally, electroantennogram recordings show that the differences in the olfactory sensitivity are already a result of differences in "whole antennal" sensitivity. Larger antennae with a higher number and higher density of olfactory sensilla increase the probability to catch and detect odor molecules than smaller antennae.

We propose that the olfactory system of polymorphic bumble bee workers is a promising model to study the effect of differences in neuron number (sensory neurons, antennal lobe interneurons and projection neurons) on olfactory perception and processing as well as olfactory mediated behavior.

Lateral Inhibition in the Insect Antennal Lobe 441

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Processing within the antennal lobe modifies the final representation of odors in the assembly code carried by projection neurons to higher brain regions. The gross morphology of the insect antennal lobe is analogous to the olfactory bulb of vertebrates where lateral inhibition between neighboring glomeruli via the mitral/granule system has been found (review Shipley and Ennis). In contrast to the olfactory bulb, no clear morphological correlate to mediate lateral inhibition has been found in the antennal lobe.

However, in a calcium imaging study on honey bees it was shown that the representation of odors at the level of the projection neurons is more confined then at the level of the receptor neurons (Sachse and Galizia). We examined the mutual influence of neighboring glomeruli by recording intracellularly from projection neurons of the moth *Heliothis virescens* during single odor and binary mixture stimulation.

Constant stimulus intensity, irrespective of whether a pheromone was presented alone or in combination with a second pheromone, was achieved with four separate flow channels. Two channels were used for a continuous air flow of 2 l/min and both could be switched to two other flow channels holding the odor cartridges. Neurons werre stained after recording with Lucifer Yellow and the antennal lobe was examined as a whole mount preparation with a laser-scanning confocal microscope. This allowed us to identify the glomerulus where the investigated neuron is arborizing and the distance to the other glomeruli where pheromones are represented was measured.

We found that the responses of component-specific projection neurons were reduced when the primary component was presented as a mixture with another pheromone compound (to ~73%, n=43). The level of inhibition was constant over a wide range of concentrations of the second compound (2-2000x the 1^{o} compound). The inhibition was dependent on the distance between the two glomeruli where the two pheromones of the binary mixture were primarily represented. Inhibition was weaker as the distance between glomeruli increased (response reduced to ~58% in adjacent glomeruli vs. ~83% in separated glomeruli, n=9). These results show that odor information processing is functionally similar in the antennal lobe and the olfactory bulb. However, the mechanism of lateral inhibition differs. Lateral inhibition may be mediated by either antennal lobe local neurons or NO release by receptor neurons.

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442 Optical imaging of odorant representations in the *Drosophila* brain using the calcium sensor protein *cameleon*

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In *Drosophila*, olfactory receptor neurons located in the antennae and maxillary palps terminate within the paired antennal lobes? 43 glomeruli. The antennal lobe output neurons (projection neurons) ramify within the AL in a genetically determined way. This stereotypic organization results in odotypic maps in terms of differential and combinatorial glomerular activities. Complex signal integration within the insect AL results not only from the high degree of convergence - in *Drosophila* from ~1300 receptor neurons to 150-200 projection neurons - but also from lateral inhibitory networks that

shape both spatial patterns and temporal synchrony of PN responses. A spatial representation of odor stimuli at the PN target areas, the lateral protocerebrum and the mushroom body calyx, is as yet unknown. Of these, the calyx is of particular interest since the mushroom body is known to be involved in olfactory learning and memory, i.e. in applying an experienced relevance to a formerly neutral odor stimulus. Its mode of action however remains hypothetical. A major question is if and how different odors are spatially represented at the mushroom body input region. This question is difficult to approach using conventional neurophysiological techniques, be it electrophysiological or optophysiological recordings. Studies involving single cells allow an ensemble analysis only by comparing many cells from different individuals. Multielectrode arrays give a record of many neurons at the same time and area, but do not reveal their identity. Optical methods using conventional dyes also give access to many neurons simultaneously, but measure signals from entire neuropils containing processes of many neuron types and connections, rather than from populations of cells defined by a common identity. In this study we have established a protocol for *Drosophila* that allows us to selectively measure the activity of identified cell populations by combining transgenic and optical imaging techniques, showing that the DNA encoded fluorescent probe cameleon (1) targeted to defined cells can be successfully exploited in an *in vivo* preparation of a highly evolved brain. We report our protocols for some of the reported cameleon variants and for different imaging setups. Using this novel method we recorded spatio-temporal odor representations in projection neurons at their input region in the AL and at their output region the mushroom body calyx. We found that odorants are represented in the antennal lobe as a combinatorial, odorant specific pattern of glomerular activity, as studies on other insects have shown. In the mushroom body calyx, odorants also evoke spatially restricted, combinatorial and odorant specific patterns of activity, that rise and fall monotonically with the stimulus onset and offset, respectively. We conclude that the glomerular odor map in the antennal lobe is reshuffled into a calycal map of odor representation (2).

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Representation of behaviourally generated optic flow by blowfly neurons thought to be involved in optomotor course control

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Knowing about the wiring of a neuronal circuit does not allow us to infer safely how efficiently and reliably information is represented in natural behavioural situations. This is because the mechanisms underlying neuronal information processing usually are highly non-linear and results obtained with relatively simple stimuli as are conventio-

nally used in visual sciences can, thus, not be generalised to the usually much more complex stimuli as are characteristic of natural behavioural situations. For instance, the dynamics of natural optic flow is largely determined by the animal's self-motion and thus may have peculiar properties. Flying blowflies usually change their direction of flight by brief and rapid body saccades during which the blowfly may acquire angular velocities of up to 4000 deg s^{-1}.

We analyse how motion-sensitive tangential cells (TCs) in the 3rd visual neuropile respond to optic flow that blowflies encounter during free flight. Recent development of miniature magnetic sense coils that can be carried by flying blowflies now make it possible to measure flight trajectories accurately in three dimensions (Schilstra & van Hateren 1998, J Neurosci Meth 83, 125-131) and to reconstruct the optic flow that is experienced by the moving animal. Moreover, these stimuli can then be replayed to the blowfly at a frame rate of 370 s^{-1} by a specially designed stimulus device ('FliMax') while activity of TCs is recorded (Lindemann et al. 2003, Vis Res 43(7), 779-791). In this way it is possible to analyse for the first time how natural optic flow is represented by the visual system of the fly.

As judged by its input organisation, a specific TC, the so-called HSE-cell, can be expected to respond best during rotations of the animal about its vertical body-axis. The responses of the HSE-cell as well as of one of its prominent input elements (H1-cell) to optic flow experienced during free-flight only partly fit these expectations. Although the neurons respond to saccadic turns of the animal, the responses may be considerably affected by the translational optic flow during the flight sequences between the saccades. This could be shown by manipulating the distance to environmental objects in the behaviourally generated optic flow. Hence, between saccades the TCs provide with their responses information about the three-dimensional layout of the environment, although the analysed neuronal circuit was expected, on the basis of its responses to conventional stimuli, to provide mainly information about body rotations.

This finding emphasises that the significance of neuronal circuits can only be assessed if they are probed in their natural operating range.

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444 Flight performance modified by environmental changes in the blowfly *Lucilia*

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Saccade-like turns in flight, i.e. fast changes in body orientation, are a characteristic of the locomotor behaviour of flies. It is not yet clear what leads to the initiation of a saccade, but "optic flow", the constantly fluctuating pattern of visual input that is experienced by animals in movement, is very likely involved in the process.

In free flight, saccades appear to be initiated randomly, as the fly's fancy dictates, which makes it difficult to draw conclusions as to the reasons at their origin. In a series of behavioural experiments, we sought to spatially restrict the area in which a high proba-

bility of saccades can be expected and to shift this area in space by varying the textural properties of parts of the surroundings. To this end, a flight tunnel was specially designed to include two obstacles covered with a random-texture pattern. This configuration forced the flies to follow an S-curve. Freely flying blowflies (genus *Lucilia*) were filmed in the tunnel by two orthogonally arranged high speed cameras (sampling rate 500Hz). Three-dimensional reconstruction of the flies' trajectories and body long-axis orientations made a detailed analysis of the flight behaviour and a precise localisation of saccade positions possible.

We modified the size of the random-texture elementary units covering the furthest obstacle and analysed flight trajectories and saccade characteristics. As pattern size decreases, trajectories become more angular and the turn that is necessary to avoid the second obstacle takes place later on average. Saccades are less well localised and take place closer to the obstacle, which seems to be recognised later as a hindrance. Flies make use of the whole height of the tunnel, independently of the pattern condition, with the average flight taking place around the middle of the tunnel.

Saccades were classified according to their maximum rotational velocity. "Slow" saccades (rotation velocities between 750 deg s^{-1} and 2000 deg s^{-1}) are much more numerous than "fast" ones (rotation velocity superior to 2000 deg s^{-1}). They take place relatively indiscriminately along the flight path, whereas the fast saccades are mostly localised before a distinct change in flight direction. The translational velocity of flies was found to remain constant in a 140ms time window around the peak of slow saccades. In contrast, for fast saccades, the translational velocity decreases noticeably during the saccade.

We have shown that targeted modifications of the fly's surroundings lead to changes in flight behaviour and saccade production in the flight tunnel. The tunnel thus proves to be a useful tool in the endeavour to determine how optic flow contributes to saccade generation. We will analyse which optic flow features play a role in eliciting saccades and how motion-sensitive neurons in the fly respond to these relevant stimulus features.

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Processing of behaviourally generated optic flow: Model simulations

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The visual motion processing system of the fly has been systematically analysed in great detail. This system analysis led to the conclusion that retinal image velocity is encoded in a highly nonlinear and ambiguous way. The response of wide-field motion-sensitive neurons, the so called tangential cells (TCs), does not only depend on velocity, but also on the spatial statistics and the contrast of the stimulus as well as on the dynamics of velocity changes.

The stimuli traditionally used for system analysis are simple compared to the stimuli the system processes in a behavioural situation. Furthermore, it is not possible on the basis of artifical stimuli to infer on the natural operating range of the system. Nevertheless, it

is often implicitly assumed that the nonlinearities found in system analysis may be irrelevant for the animal in a natural situation, and that the motion vision system enocdes the time course of visual motion veridically.

However, recent experiments with optic flow stimuli as were experienced by freely walking flies revealed, that TCs are operating far beyond their linear range under behaviourally relevant conditions. However, the responses were remarkably invariant to changes in the spatial layout and in the density of the textures of the surround, as long as the animal was not too close to the objects in its environment.

At first sight, this finding appears to contradict previous results obtained with conventional motion stimuli. Nevertheless, a model of the fly's visual motion pathway is capable of reproducing the previous results as well as the responses to behaviourally generated optic flow in great detail.

The model consists of an algorithmic model of local motion detectors of the correlation type that receive input from a linearised spatiotemporal filter and simulates the peripheral visual elements. A simplified membrane equation implements the nonlinearity found in the spatial integration of TCs. By simplifying this model stepwise, we analysed which parts are necessary for the specific properties of the system as they manifest themselves during stimulation with behaviourally generated optic flow.

One unexpected result of the experiments was that the membrane potential of the cell flips between two states of relatively constant de- and hyperpolarisation. This property can be reproduced even with the most simplified model, a linear summation of an array of motion detectors.

The nonlinear integration by TCs causes the relative independence of the response amplitude from texture density. However, this invariance is obtained only, if the peripheral preprocessing removes the absolute luminance information from the input signal to a large extent.

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446 Behavioural resolution of the honeybee eye is limited by the optical resolution of border detectors.

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By the standards of camera types eyes the resolution of insect compound eye is poor. For example, the optical resolution of the bee eye is about 100 times coarser than our own. Thus one would expect behavioural resolution of the honeybee vision to be close to the limit set by the optics of their eyes. However, previous experiments have shown that a circular target in order to be detected must subtend an angle of at least 5 degrees, i.e. it must cover at least 7 ommatidia (Giurfa, Vorobyev, Kevan, Menzel, 1996, J. Comp. Physiol. A 178:699-709). Why don?t bees detect targets subtending smaller visual angles? We suggest that behavioural response is yielded only by stimuli that have

recognizable borders and therefore can be referred to as objects. Then detection may be limited by resolution of border detectors ? when borders of stimuli merge, stimuli would not be detected. The resolution of limits of the border detectors can be estimated from the optics of the bee eye. Predictions of the border detection model agree nicely with the results of previous behavioural experiments ? the smallest circular target whose borders can be optically resolved must subtend an angle of 5 degrees.

To test if the behavioural resolution of the bee eye is indeed limited by the optical resolution of border detectors, we trained bees to detect oval stimuli whose diameter in a longitudinal direction was 4 times longer than in a short one. The ovals were either horizontally or vertically oriented. The optical model of border detection predicted the limiting visual angle for such ovals to be 9.5 and 7.7 degrees (long diameter) for horizontal and vertical orientations respectively. In the case of horizontal orientation bees could detect an oval subtending 11 degrees (85% correct choices) but they fail to detect one subtending 7.6 degrees (51% correct choices). In the case of vertical stimuli bees easily detected an oval subtending 11 degrees (91% correct), the 7.6 degrees oval was difficult to detect (69% correct) and they could not detect an oval subtending 6.4 degrees (53% correct). Thus the results of behavioural experiments agree with the results of the model predictions, indicating that the resolution of the honeybee vision is close to the limit set by the optics of their eyes.

Robustness and fragility of information in *Drosophila* photoreceptors

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Photoreceptors must encode the vast range of spatiotemporal light intensities to which they are exposed into voltage responses of limited amplitude and speed. Thus, they must maintain coding precision in the face of changing internal and external conditions and over a broad range of activity levels. It is possible to assess their performance by calculating their information capacity, a measure of the number of states a signalling system can transmit in a given time window. How robust is this neural information? *Drosophila* photoreceptors contain an array of K^+ channels, including *Shaker* and delayed rectifier K^+ channels, which modify the light generated current. Thus, the K^+ channels play an important role in determining the activity level of the photoreceptor during a particular light stimulus. We altered the expression levels of these channels and, therefore, the activity of the photoreceptors to determine the robustness of neural information. We recorded from *Drosophila* photoreceptors *in vitro* to identify the effects of particular mutations upon the K^+ currents. These mutations included $Shaker^{14}$, $Shab^{1}$, $Shab^{2}$ and $Shab^{3}$ as well as a $Shaker^{14}Shab^{3}$ double mutant. We show that the Shab gene codes for the slow delayed rectifier K^+ channel in *Drosophila* photoreceptors. Using these mutations it was possible to experimentally manipulate specific K^+ conductances. We then

recorded from the mutant photoreceptors *in vivo* whilst presenting them with dynamically modulated light, which allowed the calculation of their information capacity. The reduction or loss of a particular K^+ conductance led to a change in the dynamics of the photoreceptor response and a concomitant change in the information capacity. The information capacity was not robust to changes in the K^+ conductances. Comparison of the experimentally determined changes in coding with a Hodgkin-Huxley type model of the photoreceptors showed that many of the effects of altered channel expression could not be predicted from the biophysical properties of the channels. Indeed, many of the coding properties of the mutant photoreceptors appeared to result from a compensation of one channel by another. These results show that neural information is fragile despite mechanisms that compensate for altered channel expression.

448 The Metabolic Efficiency of Signalling in Fly Photoreceptors

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Neural processing is constrained by the biophysical relationships between energy consumption and signal quality. Animals have only a limited amount of energy to expend on neural processing but must also extract reliable information from their environment to generate adaptive behaviour. The interplay between these two factors could influence the number and types of ion channels, and the number of transduction units in neurones, as well as their size. By recording intracellularly from insect photoreceptors *in vivo* whilst presenting them with a dynamically modulated light stimulus it is possible to measure their information capacity. Electrical signalling dominates energy usage due to the large numbers of ions flowing across the membrane that must be pumped back to maintain concentration gradients. The activity of the pump can be estimated using a biophysical model from the currents recorded *in vivo*, enabling the metabolic cost of neural information to be calculated. We investigated the relationship between the size of cells, their energy consumption and their ability to transmit information by comparing fly photoreceptors. The largest photoreceptors (R1-6, *Calliphora*) could signal at twice the bit rate of R7 photoreceptors (*Calliphora*) and four times the bit rate of *Drosophila* R1-6 photoreceptors. However, the larger cell consumed more energy because of the increased ion flux. All three photoreceptors operated most efficiently (lowest cost per bit) in full daylight. The reduction in cost per bit at bright light levels is due to the high cost of maintaining the resting potential coupled with the reduction of gain and increased bandwidth brought about by light adaptation. The cost of maintaining the resting potential appears to be largely due to the need to oppose an inward current generated by spontaneous activity in the transduction machinery. However, information rate also increases with the number of transduction units used for signalling. The basic relationships between numbers of transduction units, information capacity and metabolic cost place constraints on photoreceptor design. These results can be generalised to other neurones and to signalling with synapses and channels. The relationships promote the reduction in neural structures to the essential minimum by penalising excess capacity.

Experience-dependent plasticity, gain control and information capacity in *Drosophila* photoreceptors 449

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Experience-dependent plasticity and adaptation are important features of the nervous system, providing functional flexibility in response to varying external conditions. The visual system of invertebrates has proved to be a good model system for the study of experience-dependent changes. Most of these studies focus upon structural changes of the visual processing centres as well as upon interneurones and their synaptic connections in response to dark-rearing. Therefore, relatively little is known about experience-dependent changes within the photoreceptors.

Here we demonstrate the effects of both short and long term light exposure on the response properties of adult *Drosophila* photoreceptors to light stimuli of different intensities. Flies were reared at 19°C either under a 12:12 light/dark cycle (light-reared, LR) or in constant darkness (dark-reared, DR) and were exposed to either light or darkness several hours prior the experiment. Intracellular recordings were carried out *in vivo* from R1-6 photoreceptor cells.

The photoreceptor signalling was quantified by calculating the signal-to-noise ratio, frequency and impulse responses, and information capacity of photoreceptor voltage responses. We show that both developmental and short-term light exposure altered the response properties of *Drosophila* photoreceptors. Exposure to light during development changed the linear impulse response significantly but had little effect on the information capacity. Photoreceptors of light reared flies have shorter time-to-peak values, larger amplitudes and narrower impulse responses compared to photoreceptors from flies reared in darkness. In comparison the effects of short-term light adaptation on the impulse responses occurred only within a limited range and were biased by developmental light experience. We demonstrate that light adaptation prior to the experiment reduces the ability to adapt to different light intensities. Furthermore, short-term light exposure increased the information capacity independent of the developmental light history. These results suggest that fly photoreceptors can be tuned to recent light exposure within a limit that is set by the developmental light experience of the fly. Such response scaling might occur to maintain a constant information capacity under fluctuating developmental conditions.

450 Munc13 proteins in the retina: Synaptic expression and function

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Munc13-1, 13-2 and 13-3 comprise a family of proteins that are essential for vesicle priming at synapses. Mice deficient in Munc13-1 lack glutamatergic synaptic transmission and are not viable. We examined the expression and synaptic localization of the three Munc13 proteins in the wild-type retina, and analysed the structure and function of the retinae of Munc13-2 and Munc13-3 deletion mutant mice.

Munc13 mRNA expression and protein localization was examined in wild-type retina by *in situ* hybridization and immunocytochemistry, respectively. Structure and function of retinae of Munc13 deletion mutant mice was studied by immunocytochemistry and recordings of ERGs.

Whereas all neurons of the retina seemed to express Munc13-1 mRNA, subsets of bipolar and amacrine cells expressed Munc13-2 and Munc13-3 mRNA, and expression was mutually exclusive. Immunocytochemistry localized Munc13-1 presynaptically at photoreceptor and amacrine cell synapses and postsynaptically in dendrites and processes of bipolar and horizontal cells, respectively. Munc13-1 was absent at bipolar cell synapses. This was also true for Munc13-3, whose expression was restricted to amacrine cell synapses and to bipolar cell dendrites. The structure and function of the retinae of Munc13-2 and Munc13-3 deletion mutant mice appeared normal.

The three Munc13 proteins are heterogeneously expressed at retinal synapses. Photoreceptor ribbon synapses express only Munc13-1, amacrine cell synapses express combinations of Munc13-1/13-2 or Munc13-1/13-3, and bipolar cell ribbon synapses appear not to express any of the three Munc13 proteins. In addition to presynaptic localization, we also identified a postsynaptic site of action of Munc13 proteins at retinal synapses. As the lack of Munc13-2 and Munc13-3 in deletion mutant mice has no obvious effect on the structure and function of the retina, Munc13-1 is the key functional Munc13 protein at retinal synapses.

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Morphological alterations in the retina of CNG3 $^{-/-}$ / Rho $^{-/-}$ double mutant mice

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Purpose: Due to the lack of both functional cones (deletion of the CNG3 channel) and rods (absence of rod opsin), CNG3 $^{-/-}$ / Rho $^{-/-}$ double knockout (DKO) mice are unable to sense light with their photoreceptors. By comparing the retinal morphology of these animals with single knockout and wild-type animals, we can examine the role of functional photoreceptors in postnatal development.

Methods: Shortly fixed tissue (4% paraformaldehyde, 15 min) was processed for immunofluorescence labeling using antibodies against blue opsin, calbindin, PKCalpha, NK3R, bassoon, mGluR6 and GluR1. For electron microscopy, longer fixed tissue with 0.5% glutaraldehyde was used.

Results: The absence of photoreceptor function in DKO mice was documented by the lack of any electroretinographic (ERG) responses. Microscopically, rod degeneration begins at about postnatal week (PW) 4 and leads to an almost complete loss of both rods and cones by PW12. Between PW4 and PW7, cones as well as horizontal cells and rod bipolar cells undergo a remarkable process of neurite sprouting. In the outer plexiform layer (OPL), the number of rod terminals decreases, but the sur-viving ones show an increase in the number of the synaptic ribbons and postsynaptic elements. Postsynaptic receptors like GluR1 and mGluR6 are synaptically clustered until PW7. Morphological alterations in the inner retina appear at later stages; until PW10 the inner retina appears normal.

Conclusions: CNG3 $^{-/-}$ / Rho $^{-/-}$ DKO mice show a progressive degeneration of all photoreceptors within three months after birth. However, during the first weeks until PW7, presynaptic markers and postsynaptic glutamate receptors are expressed in the OPL, suggesting that neurotransmission could take place. This indicates that functional photoreceptors are not required as a developmental stimulus up to this point.

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452 Immunocytochemistry reveals the molecular composition of first and second order visual synapses in the locust

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The first and second order visual synapses of invertebrates share some kinetic properties. They are able to release neurotransmitter continuously and to transmit graded presynaptic potentials (reviewed in *Juusola M. et al. 1996, Trends Neurosci 19: 292-297*). In vertebrates, output synapses of photoreceptor and bipolar cells belong to the ribbon type. This type of synapse is distinguished by a specialised presynaptic ribbon near the active zone and lacks one family of presynaptic proteins termed synapsins, which are thought to be involved in regulating the number of vesicles available for release (reviewed in *Lenzi D. and von Gersdorff H. 2001, BioEssays 23:831 – 840*). In insects, graded and continuously releasing synapses involved in vision do not have any anatomical specialisation that distinguishes them from synapses that operate with presynaptic impulses. However, like vertebrate ribbon synapses, *Drosophila* photoreceptor output synapses appear to lack synapsin (*Klagges et al. 1996, J Neurosci 16: 3154-3165*). We are using immuno fluorescence and immuno electron microscopy to investigate the molecular composition of identified graded, continuously releasing synapses in the locust visual system. The first and second order synapses of locust vision appear to differ in their molecular composition. As in *Drosophila*, the first order synapse, the photoreceptor output onto second order neurons in the compound eye visual system, fails to stain with an antibody against *Drosophila* synapsin (SYNORF1, *Klagges et al. 1996, J Neurosci 16: 3154-3165*, courtesy to Dr. E. Buchner, Würzburg, Germany). In contrast, another visual synapse that transmits graded potentials, the output synapse of large ocellar (L-) interneurons in the ocellar tracts, labels strongly with the anti synapsin antibody. This finding suggests that the first and second order visual synapses differ in the way they regulate neurotransmitter release in spite of similar physiological features.

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453 Nonlinear analysis of normal and *shaker* K^+ channel knockout *Drosophila* photoreceptors stimulated by white noise and natural light signals

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We studied phototransduction in wild type and *Shaker* mutant (Sh^{KS133}) *Drosophila* photoreceptors by stimulating with white noise and natural time series of light contrast intensities while recording the resulting intracellular membrane potential fluctuations. A

second-order Volterra kernel series was fitted to the data using the parallel cascade method. The kernels were used to characterize the nonlinear dynamic properties of transduction in normal receptors and in mutants lacking functional *Shaker* channels. First-order kernels to white noise stimuli were similar in wild type and Sh^{KS133} photoreceptors, indicating that the basic light transduction machinery was always intact. However, second-order kernels of *Shaker* mutants lacked a large, early amplification, indicating a novel role for *Shaker* K^+ channels in amplifying and accelerating the voltage responses of wild-type photoreceptors to light fluctuations around a steady background level. A cascade model of two nonlinear static components surrounding one linear dynamic component (NLN model) was fitted to the same data using minimum square error procedures. The NLN model was able to reproduce the experimental responses as effectively as the second-order Volterra model for white noise stimuli. Parameters obtained by fitting the model to the experimental data suggest that normal *Shaker* K^+ channels primarily contribute an early, positive nonlinearity under white noise stimulus conditions. Responses of *Drosophila* photoreceptors to large amplitude natural light intensity fluctuations could also be well approximated by an NLN model, but with a stronger compressive nonlinearity at the end of the cascade that is probably due to membrane shunting of larger depolarizations.

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Analysis of fly phototransduction proteins by MALDI-TOF mass spectrometry

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The phototransduction cascade shared by the flies *Drosophila melanogaster* and *Calliphora vicina* is prototypical for G-protein mediated signaling mechanisms. Light is perceived by a G-protein coupled receptor (rhodopsin) and transduced into electrical signals. The visual G-protein connects the receptor rhodopsin to the phospholipase C β-mediated phosphoinositide signaling which finally results in opening of Ca^{2+} channels. The Ca^{2+} channel TRP is tethered into a macromolecular protein complex by the scaffolding protein INAD together with an eye-specific protein kinase C (ePKC) and a phospholipase C β (PLC β), the effector of the α-subunit of the visual G-protein. Besides the knowledge of the main components involved in phototransduction in fly photoreceptors, little is known about special mechanisms regulating single steps of the signaling cascade like the phosphorylation of rhodopsin, of the scaffold protein INAD, or of the ion channel TRP. The association of further components to the INAD signaling complex like the ion channels TRPL and TRP γ or the attachment of cytoskeleton elements is discussed controversely. New approaches towards the identification of new components as well as to characterize known proteins in more detail are offered by mass spectrometric analysis of the photoreceptor proteome.

Since its invention, matrix-assisted laser desorption/ionisation mass spectrometry (MALDI-MS) has rapidly developed into a powerful peptide mapping tool for the direct analysis of peptide fragment mixtures, localization of covalent modifications, and for protein identification. In-gel digestion of polyacrylamide gel-separated proteins has proven to be an efficient method to generate these peptide fragment mixtures. However, most of the previous work has concerned soluble proteins rather than integral membrane proteins. Using a combination of 2-D gel electrophoresis and MALDI-TOF (matrix-assisted laser desorption/ionisation time of flight) mass spectrometry, we characterised proteins contained in the light-absorbing membrane of fly photoreceptor cells. The analysis of mass spectra of peptides obtained after tryptic in-gel digestion of *Calliphora vicina* rhodopsin is a main locus of these studies.

Furthermore we used mass spectrometry for the analysis of recombinantly expressed phototransduction proteins. Comparing MALDI-TOF spectra of immunoprecipitated TRP recovered from wildtype *Drosophila* heads as well as from *Drosophila* S2 cells expressing the INAD signaling complex revealed that these cells express the TRP protein in full length. The ability to express full-length TRP is a prerequisit for a functional analysis of the recombinantly expressed INAD complexes in S2 cells.

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455 Characterization of *Drosophila* mutants with defects in photoreceptor cell patterning

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The *Drosophila* compound eye is composed of 750 units called ommatidia. Each ommatidium contains 8 photoreceptors: six outer photoreceptors R1-6 expressing the rhodopsin Rh1 and two inner photoreceptors R7 and R8 expressing the rhodopsins Rh3 to Rh6 [1]. There are two classes of ommatidia containing different rhodopsin complements in their inner photoreceptor cells: *pale* ommatidia (Rh3 in R7 and Rh5 in R8) and *yellow* ommatidia (Rh4 in R7 and Rh6 in R8) which are stochastically distributed throughout the *Drosophila* compound eye at a ratio of about 30% *pale* and 70% *yellow* ommatidia [1]. The molecular mechanism, that regulates the distribution of *pale* and *yellow* ommatidia in the *Drosophila* compound eye and the coordinated expression of either Rh3 and Rh5 or Rh4 and Rh6 in adjacent R7 and R8 cells is largely unknown. The finding that, in absence of a R7 photoreceptor cell, Rh5 expression is abolished in all R8 cells which now express Rh6 instead, has led to the hypothesis that a signal from the Rh3-expressing R7 cell is responsible for Rh5 expression in the underlying R8 cell, and that the expression of Rh6 in the R8 cell represents the default state [1].

In order to identify genes involved in rhodopsin patterning in the *Drosophila* eye, we performed EMS mutagenesis screens to disrupt normal rhodopsin expression in inner photoreceptor cells. We mutagenized flies expressing the reporter gene β-galactosidase under control of the *Rh5* promotor to monitor changes in Rh5-expresssion via X-gal staining in whole mounts. Mutant lines showing abnormal β-galactosidase expression in pale ommatidia, i.e. either more or less β-galactosidase expression than wild type, were

analysed applying histochemical and molecular biological methods. For example, in one mutant fly which showed very little β-galactosidase staining, Rh5 expression in R8 cells was abolished and nearly all R8 cells expressed Rh6, reminiscent of a fly lacking the R7 photoreceptor cell. Several mutant lines with enhanced β-galactosidase staining expressed both rhodopsins (Rh5 and Rh6) in the same R8 cell, possibly due to a lack of repression of Rh6 expression. This was accompanied by an increase in Rh3-Rh6 mispairing in R7 and underlying R8-cells. In addition, some of these mutant lines expressed the Rh5 reporter gene in other R8 cells than those which contained Rh5 itself. Thus, mutational disruption of the wild type rhodopsin expression pattern creates ommatidia within a single eye showing mispaired R7 and R8 rhodopsins, photoreceptors that contain two rhodopsins, and separated expression of the Rh5 reporter gene and Rh5 itself.

Taken together, these findings suggest that the regulation of rhodopsin expression in inner photoreceptor cells of the *Drosophila* compound eye is not governed by a simple and final decision between pale and yellow ommatidia alone. Instead, it seems to be a finely tuned mechanism which results in a number of unexpected expression patterns when disrupted.

[1] Chou,W.H.; Huber,A.; Bentrop,J.;

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Visual differences: The function of rhodopsin phosphorylation in *Drosophila* photoreceptors 456

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For many G-protein coupled receptors (GPCRs), including vertebrate rhodopsin, phosphorylation at serine and threonine residues in the C terminal tail domain is part of the mechanism which controls the deactivation of the active state. In *Drosophila* photoreceptors, however, there is no evidence that phosphorylation of rhodopsin triggers a deactivation mechanism. In particular it has been shown for the major *Drosophila* rhodopsin Rh1, that a deletion in the Rh1 gene, which removes all putative phosphorylation sites from the C-terminal domain of Rh1, does not impair the termination of the light response (1;2). Termination of the active M-state of *Drosophila* Rh1 rhodopsin is instead triggered by the binding of members of the arrestin family of regulatory proteins.

In the present study, designed to investigate the function of rhodopsin phosphorylation in *Drosophila*, we eliminated the six putative phosphorylation sites of Rh1 altogether or one by one. After introducing targeted point mutations, the effect of each mutation was tested *in vivo*. Transformation of phosphorylation site-mutated rhodopsin into a Rh1-null mutant functionally rescues the wild type. Thus, phosphorylation of these sites is without relevance for 1) targeting and transport of rhodopsin to the rhabdomeral compartment, 2) retinal degeneration 3) the ability of rhodopsin to trigger phototransduction, 4) spectral shifts recorded after photoconverting rhodopsin from its P- to the M-state, and

most importantly 5) the deactivation of metarhodopsin by binding of Arrestin2.Taken together there is no experimental evidence neither from earlier (1;2) nor from the present study that phosphorylation of *Drosophila* rhodopsin is functionally related to the rapid termination of photoresponses. This constitutes a distinct difference to the role of rhodopsin phosphorylation in response deactivation in the vertebrate photoreceptor.

Concerning the function of Rh1 phosphorylation, we show that the light-induced degradation of metarhodopsin is phosphorylation-dependent. Under light conditions which establish a high content of metarhodopsin within the photoreceptor cell, the total amount of visual pigment decreases in wild-type flies, not in flies expressing phosphorylation-deficient rhodopsins. The difference between wild-type and mutant flies is most obvious within 24 hrs following the onset of light. Over a period of days, visual pigment breakdown reaches the same level in the mutant as in the wild type. This suggests the existence of two autonomous degradation pathways for metarhodopsin, a quick, phosphorylation-dependent mechanism, which may utilize an ubiquitin-dependent degradation machinery, and a slower, phosphorylation-independent mechanism, which may proceed via the arrestin-clathrin route. In *Drosophila* photoreceptor cells, the removal of "used" visual pigment molecules from the photoreceptive membrane is accomplished though an internalization mechanism, whereas vertebrate photoreceptor cells undergo a shedding of photoreceptive disc membranes into the phagocytotic environment of the retinal pigment epithelium. It appears that in this context the function of visual pigment phosphorylation is shifted from inactivation (vertebrates) to a regulator of internalization (invertebrates).

457 The INAD signaling complex of *Drosophila* photoreceptors: Assembly and characterization in a cell culture system

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Assembly of multiprotein complexes is a ubiquitous phenomenon involved in functions like signal transduction (*Drosophila* phototransduction), establishment of cell polarity (*C. elegans*: LIN-complex in polarized vulva epithelial cells), and formation of neuronal synapses. In *Drosophila* phototransduction the clustering of the INAD signaling complex is believed to increase specifity of signaling by preventing cross talk between different signaling cascades as well as to accelerate the speed of signal transduction (Huber et al. (1996) EMBO J. 15: 7036-7045; Chevesich et al. (1997) Neuron 18:95-105; Tsunoda et al. (1997) Nature 388: 431-440). The major components of this complex comprise a phospholipase Cß (PLCß), an eye specific protein kinase C (ePKC), a cation channel (TRP) and the scaffold of the complex, INAD. Refering to the scaffolding protein this complex is called the INAD signaling complex. Its correct localization in the rhabdomeral photoreceptor membrane and the stability of the core proteins depend on the proper assembly of the signaling complex, but little is known about the site and the mechanism of the assembly process.

We established the INAD signaling complex of *Drosophila* in a cell culture system by coexpressing the four major complex components in Schneider 2 (S2) cells. In selected

cell clones we were able to show coexpression and colocalization of the four major components INAD, PLCß, ePKC, and TRP by immunocytochemistry. Co-immunoprecipitation experiments revealed that the INAD signaling complex is assembled after recombinant expression of its components in S2 cells. We conclude, that the INAD signaling complex is established in S2 cells by self assembly of its major components without the requirement of additional photoreceptor specific components.

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Light-regulated ion channel relocation in photoreceptor cells of *Drosophila melanogaster* - a TRPL-eGFP reporter gene study 458

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TRP (transient receptor potential) and TRPL (transient receptor potential-like) proteins form two classes of cation channels which are activated in *Drosophila* phototransduction. TRPL but not TRP has been reported to translocate back and forth between the signalling membrane and an intracellular compartment in a light-dependent manner (Bähner et al. 2002, Neuron 34, 83-93). In dark-adapted flies TRPL is present in the rhabdomeres whereas in light-adapted flies most of the TRPL is located in the intracellular compartment.

We generated transgenic flies by P-element mediated germline transformation expressing a TRPL-eGFP fusion protein under the control of the Rh1 (rhodopsin1) promotor. In order to visualize and investigate the relocation of TRPL the transgenic flies were characterized by Western Blot analysis and fluorescence microscopy. We could verify that these flies express the TRPL-eGFP fusion protein and that it undergoes a light-dependent translocation between the rhabdomeric membrane and the intracellular compartment as does native TRPL.

By quantifying the TRPL-eGFP fluorescence inside and outside the photoreceptive membrane we determined that orange light illumination results in the most efficient internalization of TRPL and we investigated the time courses of removal and reincorporation of TRPL-eGFP into the photoreceptive membrane.

In conclusion, the TRPL-eGFP reporter gene is suitable to study light-dependent TRPL relocation in the living fly and will thus greatly aid the investigation of *Drosophila* mutants which show defects in this neuronal plasticity mechanism.

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459 A double role for arrestin 1?

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Several types of arrestins occur in various signalling cascades performing different roles mostly related to G-protein coupled receptor inactivation and trafficking. *Drosophila* photoreceptors are a classical model for study of G-protein coupled signalling cascade, and two types of arrestin have been identified there, Arr-1 and Arr-2, with the role of arrestin 1 remaining unclarified. An attempt has been made to demonstrate possible participation of arrestin 1 in metarhodopsin inactivation by estimating the amount of blue light necessary to induce a prolonged depolarizing afterpotential (PDA) in arrestin 1 defficient fruitflies, a phenomenon caused by massive photoconversion of rhodopsin to metarhodopsin, the quantity of metarhodopsin largely exceeding the available amount of arrestins. Electroretinography in fruitflies raised in thoroughly controlled conditions showed that arr-1 defficient flies require only 58% of blue light to enter a PDA comparing to wild-type, white eyed fruitflies, while the results in arr-2 defficient flies agreed very well with previously published data (18% of blue light). Arr-1 mutant flies also exhibited a distinct phenotype, characterised by photoreceptor potential oscillation, very similar to the phenotype in *Drosophila* lacking TRPL channel. Moreover, both arr-1 and trpl mutant flies showed a similar light adaptation deffect: dynamic range of their photoreceptors was reduced by approx. 1 log unit, and the Hill slope of the V-log(I) curve was significantly increased comparing to wild type. Therefore, two putative roles of arrestin 1 remain to be determined by further investigation. First, its binding to metarhodopsin is probably required to terminate phototransduction, as is the case in arr-2. Second, its presence probably determines the functional interaction of TRPL channel with other components of the signalling cascade.

460 The Relative Importance of Olfaction and Vision in a Diurnal and a Nocturnal Hawkmoth

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Different sensory cues can be used to detect flowers. In different environments, one modality may be prioritised over others. Von Frisch (1919) discovered that honeybees use both odour and colour to locate flowers when they are close but only the colour from a larger distance. The relative importance of vision and olfaction has recently been investigated in the nocturnal hawkmoth *Manduca sexta* (Raguso et al. 2002). The two types of stimuli were tested alone and in combination. They found that both stimuli were necessary to attract the moths. The presence of odour elicited more hovering and the moths passed closer to the paper flowers than without odour.

We tested a diurnal and a nocturnal species of hawkmoth in a wind tunnel (3 m long, 1 m wide and 1 m high) with visual stimuli (blue or yellow disks) and odour. Tests were performed with either flowers or odour or with both flowers and odour that were pre-

sented at 20-25 cm distance from each other so that the animals were forced to make a choice between the two stimulus types. The moths were released at the side opposite to the stimuli at the beginning of each trial, and we scored the number of approaches where the moth was less than 2 cm from the stimuli.

In experiments with both olfactory and visual stimuli, 40% of the diurnal *Macroglossum stellatarum* selected the visual stimuli, and only 9% chose the odour (n=43). In contrast, only 4% of the nocturnal *Delephila elpenor* selected the visual stimuli and 58% chose the odour (n=48). There is a significant difference in the behaviour between the two species (T-test, $P<0.001$).

Experiments with only one of the stimuli present showed how the type of stimulus influences the choice to approach the stimulus. 52 % of the *M. stellatarum* approached when only the visual stimulus was presented, (n=31) and 31% when only the odour was presented (n=13). 14% of the *D. elpenor* approached the visual stimuli (n=7) and 80% with the odour (n=5).

When *M. stellatarum* detected the visual stimuli and approached them, they always extended their proboscis and touched the flower. In contrast, *D. elpenor* never extended their proboscis in any of the experiments. The results clearly prove that the *M. stellatarum* relies mainly on the visual stimuli whereas *D. elpenor* is more attracted by the flower odour, indicating that different weight is given to the two sensory channels as a result of a diurnal or nocturnal life-style.

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First evidence of orientation to the polarisation of the moon-lit sky

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The polarisation patterns of a moon-lit night sky are practically identical to that of a sun-lit sky. Whereas many diurnal animals are well known to perceive and orient to the skylight polarisation patterns, nocturnal orientation to this cue remains a rarely investigated topic. We here present the first evidence for orientation to the polarisation of the moon-lit sky, and identify the receptors used to perceive this skylight cue.

The dung beetle *Scarabaeus zambesianus* starts to fly around sunset with the aim of finding fresh dung. Once found, it forms a ball of dung and rolls it off at high speed, in a line as straight as the terrain will allow. This is done to avoid competition at and around the food source. The ball is finally buried in a suitable place to be consumed in secure solitude. While rolling, the beetle has to rely upon some sort of reference to stay on route.

A polarising filter with a circular diameter of 42 cm was mounted in a holder with four symmetrically placed legs. In the shade of the rising moon, the filter was placed over the expected path of the beetle. The e-vector transmission axis of the filter was oriented 90° to that of the skylight polarisation pattern. Thus, as the beetles (n=22) entered the area below the filter, the polarised light pattern of skylight appeared to turn by 90°. The beetles continued to roll along their set course, until they were at least 5 cm in under the filter. The beetles then turned in response to the rotated polarisation pattern. An average turn of 80.6° ± 13.4° was close to the expected 90°. All trials were made after astronomical twilight when the sun no longer contributes to the illumination of the sky. As controls, both an empty holder, and one with the polarising filter oriented parallel to skylight polarisation, were placed over the expected path of the beetle. A small turn of less than 7° was recorded in response to the two controls.

The eyes of *Scarabaeus zambesianus* are divided into separate dorsal and ventral parts. No screening pigments, but tapetal tracheoles can be found between the rhabdoms as far as half way up the rhabdom in both the dorsal and ventral eye. Microvilli from seven of eight retinula cells form the rhabdom in both eye parts, but the arrangement of these structures varies across the eye. In the top half of the dorsal eyes the microvilli of the seven cells run in only two directions oriented 90° to each other. The microvilli are well aligned along the length of the rhabdom. These two characteristics of the rhabdoms well satisfy the requirements for a polarisation opponent analyser.

462 Spatial summation in the visual system of a remarkable group of nocturnal bees

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The reasons for nocturnal activity are most easily seen in tropical areas of the world, where animals face severe competition for food. In Panama, the benefits of reduced competition and lower predation risk at night have driven a remarkable group of solitary halictid bees (*Megalopta* spp.) to become nocturnal.

Throughout the world, the vast majority of bees are day-active and all of them have apposition compound eyes, an eye design adapted for bright daylight. Nevertheless, the Panamanian bees have retained these eyes during their transition from day to night despite being highly unsuitable for nocturnal vision. Apposition eyes have small lenses and therefore a poor photon catch, resulting in unreliable visual signals at low light intensities. According to theoretical calculations, these apposition eyes should render nocturnal bees blind by mid dusk. However, far from being blind, the Panamanian bees forage at night in starlight intensities. Theoretical work on nocturnal bees suggests that nocturnal animals with small eyes use a strategy of photon summation in space in order to capture more light. This mechanism probably involves the neural summation of signals originating from large groups of ommatidia. Consequently, this raised the question which cells are responsible for spatial summation.

By using a combined Golgi–electron microscopy method (Ribi, 1976), we investigated the neuronal organisation of the lamina, the first neuropil of the optic lobe, where spatial summation most probably takes place. The cells involved in spatial summation are most likely of the lamina monopolar cell type, of which some feature wide horizontal arborisations in nocturnal halictids. In order to interpret the results of the nocturnal bees correctly, we studied the lamina anatomy from a closely related diurnal group of halictid bees (*Lassioglossum leucozonium*). Unlike the nocturnal bees, no extensive horizontal branching was seen in diurnal bees, in agreement with previous studies on the honeybee (Ribi, 1975).

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Structures supporting light - dark adaptation in the compound eye of Ascalaphus (*Libelloides macaronius*)

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A few decades ago Ascalaphus was used as an prominent model system for the study of UV vision in insects. Previous serial measurements of optical properties of the omatidia in the frontal part of the compound eye indicated that the optical transmission changes upon light adaptation. The migration of pigments in retinal supporting cells has been suggested as the structural basis for this process.

Our present data obtained by light and electron microscopy do not support this hypothesis. We did not detect any massive pigment migration in primary pigment cells of the Ascalaphus compound eye, neither in the proximal nor in the distal part of the omatidia. In addition, our experiments indicated that pigment grains in secondary pigment cells did also not migrate in dependence on light.

Based on our microscopical and molecular data in the frontal part of Ascalaphus eye we suppose a pupile mechanism at the tip of cristaline cone of the omatidia. Our analysis indicate that the pupil is formed as a functional unit, composed of the cristaline cone, the primary and the secondary pigment cells in conjunction with the most distal part of seven of the eight retinula cells. We gathered evidence that the closing mechanism of this functional diafragma is operated by the retinula cells. During light adaptation the most distal parts of retinula cells contract and in turn the pigment cells move towards the tip of cristaline cone. The expansion of the apical portion of the retinula cells should lead to the reverse process and to “pupil” opening in omatidia of the Ascalaphus compound eye. In a last set of experiments, we have analyzed the cytoskeletal elements which participate in these motile processes.

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464 Role of the microvillar membrane in electrical properties of insect photoreceptors

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Absorption of light quanta by rhodopsin molecules and the following phototransduction takes place in a highly convoluted membrane structure, the microvilli, in insect photoreceptors. Phototransduction leads to opening of Ca^{2+} and Na^{+} permeable ionic channels located in the microvilli. This means that, in effect, light causes current to be injected into the non-microvillar part of the photoreceptor, creating changes in the membrane voltage, which are subsequently conducted to the output part of the cell, the axon. The large membrane area of the microvilli forms a large capacitor, increasing the time constant of the photoreceptor, and effectively low-pass filters the signals (Weckström et al., J Physiol. 440: 635-657, 1991; Weckström M & Laughlin, Trends Neurosci. 18: 17-21, 1995).

The electrical coupling between the microvilli and the (presumably) isopotential non-microvillar membrane was studied by intracellular recordings and single-electrode clamp techniques in *Calliphora* photoreceptors. The small dimensions of the single microvillus, and the large total area of all the microvilli may be expected to lead to an asymmetrical situation, where light-gated currents are well conducted to the non-microvillar membrane, but currents injected via the recording electrode reach the microvilli only partially. This was also found to be the case. The reversal potential of the light-gated conductance was in current and voltage-clamp experiments much too positive to be compatible with the known properties of the phototransductive channels and the ionic composition of the fluids. The same effect is also seen in measurements of the cell impedance, where the apparent contribution of the light-gated conductance is too small to cause the recorded depolarizing responses. On the basis of the experimental results we constructed a mathematical model of the photoreceptor soma, including the microvilli. With the model we were able to quantify the reciprocal currents to and from the microvillar part of the photoreceptor, and also to find a description for the nonlinear interactions in the coupling.

465 Information processing during light adaptation in blowfly photoreceptors

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The light intensity levels during the day-night cycle can range over eight orders of magnitude. To code this large variation in dynamics to a limited range of membrane voltages, the photoreceptors adapt their properties as a function of prevailing illumination. Experiments were carried out in *Calliphora* photoreceptors because of strong experimental and theoretical background established in earlier works. Although many aspects

of adaptation have been intensively studied the role of different components of signalling, i.e. the light induced conductance and the photo-insensitive membrane, and their effect on coding and information capacity during adaptation are not known. To study this we combined *in vivo* intracellular single electrode current and voltage clamp recordings with modelling. We started by stimulating photoreceptors with steps of light in different light backgrounds and investigated the light induced component in responses. This was done by removing the effect of photo-insensitive membrane predicted by our model from the experimental data. To ensure that our method could be used to pull out the role of light currents, the potassium channel blocker TEA was injected to photoreceptors and currents during the presentation of light steps were recorded. Results validate our approach and allow us to estimate the light induced currents with the stimuli during which the voltage clamping is not possible. The effect of adaptation onto coding and information capacity was studied by using preceding light stimulation producing different time scales and amounts of adaptation followed by the test stimulation sequences (e.g. white noise) suitable for our analysis. By combining the experimental results with the modelling we could distinguish different components of photoreceptor adaptation and analyze their properties and role in information processing.

Some factors affecting the electroretinogram of the cuttlefish 466

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The eyes of the cuttlefish, *Sepia officinalis*, although similar to vertebrate eyes in gross morphology, are more comparable with other invertebrate eyes at a cellular level. To understand visual processing in cuttlefish, we investigated some of the factors that influence the retina's responses to light e.g. stimulus intensity, wavelength, etc., using recordings of the electroretinogram (ERG). Similar work has been carried out on a large variety of other species and is available for comparison. The project aims to link the physiological response characteristics of the eye with visual performance, behavioural data from the same species.

All experiments were carried out on retinas excised from humanely killed animals; these ranged in mantle body length from 2 to 14 cm. Excised retina pieces (1 cm^2) or slices (300 μmeters thick) were maintained in a perfusion bath and flash stimulated using an LED; background illumination from a white light source was added when required. The intensity of both light types could be varied using neutral density filters. The factors investigated here were the changes in ERG resulting from changes in the intensity, wavelength and duration of the stimulating light flash, the effect of background illumination, and whether these response parameters changed with the growth of the animals (larger animals have longer photoreceptors).

A single flash of light resulted in brief positive, monophasic voltage fluctuation in recordings obtained from the inner segment region of the retina. The amplitude of the ERG response increased with increasing flash intensity, generally up to a plateau level where further increases in stimulus intensity did not result in further increases in ERG amplitude. By varying flash duration it was found that, for a given flash intensity, a 10 ms duration flash was not long enough for the cells to reach maximal output, while a 500 ms flash bleached the cells, necessitating a considerable period (>5 min) without

stimulation before full recovery was obtained. Of the different flash durations tested, a 50 ms flash usually gave the largest ERG response. When the background light was quadrupled, the V/log I curve shifted 2.5 log units to the right. No significant differences were found in the absolute sensitivities and the V/log I curves obtained from the retinas from different sized cuttlefish. This was surprising, but not unprecedented. Further experiments demonstrated that the cuttlefish retina was 7 times more sensitive to blue (peak emission = 500 nm) than to yellow (peak emission = 590 nm) monochromatic light. This was predictable, as the peak absorbance of Sepia rhodopsin is 492 nm. A similar pattern of responses has been reported from other species, both vertebrate and invertebrate, and further experiments are currently in progress into the basic mechanisms of cuttlefish vision with the aim of constructing a model of their visual processing.

467 A light-microscopical probe for rhabdomere twisting in the *Drosophila* compound eye

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In the compound eye of dipteran flies, the rhabdomeres, the light-receptive structures composed of densely packed microvilli, are twisted about their longitudinal axes. This phenomenon, termed rhabdomere twisting, reduces or even eliminates the polarization sensitivity of the visual cells.

So far, rhabdomere twisting has been analysed in detail only in the blowfly *Calliphora vicina*. The present study examines rhabdomere twisting in the compound eye of *Drosophila melanogaster*. In the distal half of the retina, the rhabdomeres of the visual cells R1-R6 are tilted by about 10-30° towards the R3/R4 interface. In the lower half of the retina, the R1-R6 rhabdomeres point congruently into the other direction, away from the R3/R4 interface, and the tilt angle is larger, measuring about 45-75°. Thus, the microvilli on the upper and the lower half of the visual cell point to opposite sides of the cell and are oriented almost perpendicularly to each other.

Up to now, rhabdomere twisting could be analysed only by electron microscopy. This technique is rather laborious and thus unfavorable for addressing developmental and cell biological aspects of rhabdomere twisting and for screening for mutations that effect this phenomenon. For this reason, we searched for an alternative, light-microscopical method. We show that an antibody against phosphotyrosine (PY) can be used as a marker for rhabdomere twisting. On cryostat sections through the *Drosophila* eye, anti-PY labelled the stalk domain, the membrane domain next to the rhabdomere. In each of R1-R6, only one side of the cell was labelled, and, within the same cell, opposite sides were stained in the proximal and distal half of the retina. Comparison of the anti-PY labeling pattern with the microvillar tilt direction implies a tight correlation between both phenomena; anti-PY immunoreactivity resides always on the side the microvilli turn away of. It may thus be concluded that rhabdomere twisting is manifested not only in a morphological asymmetry but also in a molecular polarization of the cell surface.

Using anti-PY as a probe for rhabdomere twisting, we currently examine whether this type of planar cell polarity depends on the *frizzled/dishevelled* pathway. These genes are known to regulate other examples for planar cell polarity in *Drosophila*, such as the uniform orientation of cuticular hairs and bristles.

Evidence for a Polarization Compass in Monarch Butterflies 468

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Monarch butterflies, *Danaus plexippus*, are well-known for their migratory capabilities, The Eastern group of the North-American population makes an annual migration that takes them from Central Mexico to the Great Lakes and back; individual butterflies may cover distances of up to several thousand kilometers [1]. Although their migration is well-described in the literature, the sensory basis of their impressive navigational performance is only scarcely studied. One sensory cue that may help the Monarchs find their way during their long journeys is the polarization pattern of the sky.

Using both light and electron microscopy, we have examined the dorsal rim area (DRA) of Monarch compound eyes for structural specializations indicating polarization vision [2]. We found that the three dorsal-most rows of ommatidia exhibit the typical traits of an eye region dedicated to the detection of polarized skylight, as previously described for other insects [2]. The microvilli of the dorsal rim ommatidia are straight and well aligned along the rhabdom, indicating high polarization sensitivity of the photoreceptors [2,3], whereas the microvilli of the regular, unspecialized ommatidia are often curved. The dorsal rim ommatidia contain just two, orthogonally-oriented microvilli orientations indicating a polarization antagonism [2,3], whereas 3 to 4 microvilli orientations occur in the unspecialized ommatidia. These findings strongly suggest that navigating Monarchs do indeed rely on a polarization compass.

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How Owls Structure Visual Information 469

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Recent studies on perceptual organization in humans claim that the ability to represent a visual scene as a set of coherent surfaces is of central importance for visual cognition. We examined whether this surface representation hypothesis (Nakayama et al., 1995) generalizes to a non-mammalian species.

Our interest in this comparative question originates from recent studies in the barn owl showing that these nocturnal hunters view their surroundings much the same way as humans and primates do. That is, owls posses stereoscopic depth perception and are

susceptible to visual illusions (van der Willigen, Frost and Wagner, 1998; Nieder and Wagner, 1999). These findings are remarkable, because the owl's eyes are quite different when compared to that of primates, and there are also large variations in structures in their brains that interpret incoming visual information (Medina and Reiner, 2000).

Discrimination-transfer combined with random-dot stimuli provided the appropriate means for a series of two behavioural experiments with the specific aim of (1) obtaining psychophysical measurements of figure-ground segmentation in the barn owl *(Tyto alba*), and of (2) determining the nature of the information involved.

In experiment 1, two owls were trained to indicate the presence or absence of a central planar surface (figure) among a larger region of random-dots (ground) based on differences in texture. Without additional training, the owls could make the same discrimination when figure and ground had reversed luminance, or was camouflaged by use of uniformly textured random-dot stereograms. In the latter case, the figure stands out in depth from the ground when positional differences of the figure in two retinal images are combined (binocular disparity).

In experiment 2, two new owls were trained to distinguish 3D objects from holes using random-dot kinematograms. These birds could make the same discrimination when information to surface segmentation was unexpectedly switched from relative-motion to half-occlusion. In the latter case, random-dot stereograms were used which provided the impression of stratified surfaces to humans by giving unpairable image features to the eyes.

The ability to use image features such as texture, binocular disparity, relative-motion and half-occlusion interchangeably to determine figure-ground relationships suggests that in owls, as in humans, the structuring of the visual scene critically depends on how *indirect* image information (depth-order, occlusion contours) is allocated between different surfaces.

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470 Adaptive gain control in insect motion detection

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Studies in a wide range of animals suggest that contrast gain controls are common in early visual processing. In recent papers [1,2,3], we used physiological recordings from fly HS neurons to show that several motion adaptation phenomena observed in our own and earlier experiments result from neural afterimage effects (temporal high pass filtering) combined with a contrast-dependent gain control mechanism. While behavior suggests

that insects are capable of estimating angular velocity in moving patterns, sensitivity to contrast is a fundamental ambiguity for velocity coding in simple motion detector models such as the Hassenstein-Reichardt correlation model. We used electrophysiological experiments and computer modeling to investigate whether this ambiguity is resolved by adaptive gain reduction during natural image motion.

Electrophysiology: We recorded intracellularly from HS neurons in restrained hoverflies viewing a CRT. Panoramic scenes obtained from natural habitats were perspective-distorted and temporally anti-aliased (motion blurred) during animation to simulate rotational optic flow. After a prolonged (3s) initial adaptation period, 200ms bursts of test speeds were interleaved with further adapting periods to generate velocity-response curves at various combinations of contrast and adapting velocity. HS cells responded to movement across 3 decades of stimulus velocity, peaking at 100 deg. per second. The shape and magnitude of motion-adapted velocity tuning curves are robust over a large range of adapting speeds, or if global image contrast is reduced to as little as 25% of the original image. Fluctuations in the response appear to be noise in individual trials, but repeated stimulation shows much of this variability to be image feature dependent. Our results suggest that motion adaptation allows HS cells to provide reliable estimates of velocity in natural conditions, despite variability in global contrast. We propose that such 'velocity constancy' results from a non-linear gain control in motion detector inputs.

Modeling: We have modeled this gain control in an elaboration of the Reichardt Correlation model, implemented in Matlab as an array of local elementary motion detectors (EMDs). Using the same panoramic images as used in the experiments as stimuli for the model, we show that by incorporating elements to implement biological plausible processing stages such as high-pass filtering, compressive non-linearities and a contrast dependent dynamic non-linearity (either a feedforward or feedback gain control mechanism) we are able to produce very similar behavior to that observed in the fly HS neuron recordings.

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Spatio-temporal tuning for small targets from a simulated array of elementary motion detectors

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Small object detection would seem to be a fundamental process in the behavioural repertoire of most animals and we assume it to be of particular importance to the flying insects - they themselves being small objects! However, the mechanisms underlying object detection are far less understood than the wide-field systems concerned with optic flow and flight stabilization and so on. While some work has been done on the DCMD descending system of the locust and the figure-ground circuitry of the fly we wanted to

investigate if we could derive a reliable signal for small object detection using only a large array of simulated elementary motion detectors (EMDs).

We examined the responses to targets of varying size and speed. The model used a 1 degree inter-receptor angle, 1.4 degree (half-width) 2-D Gaussian blur, 100 ms pre-stimulus period (no target motion), a dark target (luminance 0) on a bright background (1.0) and an exponential delay filter (τ=35 ms).

Low-pass pre-filtering on the model inputs produced tri-phasic output signals which suggests that if EMD prefilters pass significant amounts of DC, outputs may go both above and below zero. Importantly, however, we see negative-going transients primarily for small targets moving at lower speeds: much larger (wider) targets produce positive responses only, as they cross the receptive field. The tri-phasic signal arises from a quasi 'wave interference' process whereby he residual impulse response elicited by the leading edge of the target is overlapped with the early part of the impulse response elicited from the trailing edge of the target. The mean response (DC) of this model to small targets is tiny, while the overall response power is large. Thus a band-pass filtering strategy that is selective for the negative-going transients in such responses might yield a huge gain in response selectivity for small targets. A major advantage of such a strategy is that we could design EMDs which are 'pre-selective' for target-like features even at the inputs to higher-order strategies such as the ESTMD receptive field structure discussed in earlier reports.

The response range spanned by broad domain of object sizes and speeds, evaluated on a rectangular parameter space, showed a single contour in the spatio-temporal map of the EMD array, i.e. it had an optimal size-speed tuning.

The spatio-temporal tuning map shows a diagonally oriented tuning contour that encompasses the expected tuning from small targets moving at slower speeds to larger targets moving more rapidly. An algorithm was developed to detect and quantify this tuning behavior and shown to be effective and unambiguous. The detection algorithm uses the product of the integral of the mean output response from the EMD array with the mean integral value of the rectified transient signal having an opposite polarity.

472 Ground instead of walking distances determine the direction of home vector in 3-D path integration of desert ants

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Workers of *Cataglyphys fortis* perform large scale excursions from which they return by means of path integration to their nest on a straight path. This task requires information about the lengths and the compass directions of path segments. The ants use the celestial polarization pattern as a reference for their compass. However, it is still a matter of debate from what cues the ants infer their travelled distances. Remarkably, when walking over hilly terrain, *C. fortis* obviously refers to ground distances instead of the actually walked distance (Wohlgemuth et al. 2001. Nature 411, 795). In these experiments ants were trained in a channel system over a linear series of hills. Since it was not clear

whether ants would rely on this ground distance information also in a more complex situation, we now extended the ants' task to a true 3-dimensional problem.

The basic plan of the experiment was as follows: in a channel starting from the nest, the ants were trained first for a distance *D* e.g. in a northern direction, then they were forced to walk 2 m to the east, and then 1.5 m back to the south, where a feeder was located. From the feeder the ants were transferred to a test field to observe their homing directions (they should head roughly to the west). Three groups of ants were confronted with different conditions in the first leg of this channel system. The experimental animals had to climb a 2-m long ramp up to a height of 1.65 m (angle of ascent ca. 55°, ground distance 1.15 m). Then they had to walk in a horizontal channel (at same height) to the east and the south. If the ants used the 1.15-m ground distance to calculate their home vector, they should exhibit a more northern course; if it were the actual walking distance (2 m) that counted their home paths should deviate to the south. In two controls the channels were laid on the ground, and the length *D* of the first segment was either 2 m (control A) or 1.15 m (control B). Between 24 and 30 individuals participated in each experimental situation. Homebound paths were recorded and homing directions were determined at 1, 1.5, and 2 m from the release point (see e.g. Bisch-Knaden & Wehner JEB 204, 4177).

In the main experiment (ascending ramp) the ants showed a mean vector direction that deviated by 22° to 24.4° to the north (west = 0°). This direction differed highly significantly from that of the control A (-10° to -11,2° to the south; all standard errors between 4° and 6°). The mean vector found in the main experiment was indistinguishable from that of control B (20° to 22°), demonstrating that it was not the walking distance but rather the ground distance of the ramp that determined the ants' homing directions. In this experimental design the ant's estimate of her travelling distance is translated into an angular deviation. This result provides an independent confirmation of the earlier proposal that the ants are able to assess ground distances when walking over hills, and that it is this distance estimate that is used in the ants' path integration mechanism.

Colour detection by bumblebees: Effects of target grouping 473

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The detection of coloured objects is a major visual task in foraging bees. The behaviourally determined detection limit for coloured targets in bees implicates spatial summation over several ommatidia[1] and depends on contrast distribution within the target area[2]. Here we tested the detectability of a group of coloured discs. In the honeybee detection and discrimination of coloured targets are mediated by chromatic contrast at large angular subtenses and by L-receptor (achromatic) contrast at small ones. Thus the receptive fields of chromatic detectors are much larger then those of achromatic ones.

We first tested whether bumblebees have a similar arrangement of their visual system. To that aim, individual bumblebees were trained to detect both discs with L-receptor

contrast to the background (yellow) and without it (violet). Animals of the same size (3.4-3.9 mm thorax width) were chosen in order to avoid the influence of the eye size on the detection limit[3]. The results show that the detection limits depends on the presence or absence of the L-receptor contrast. The limiting visual angles were 1.8° and 3.1° for yellow and violet discs respectively. The higher resolution compared to that of the honeybees (5° and 10° respectively) can be attributed to the larger size of the bumblebee eye.

In a second experiment bees were trained to detect a triplet of small discs arranged as a triangle pattern. The sum of areas of the three discs was equal to the area of a single disc. The yellow triplet was detected until a small disc subtended an angle of 0.75°. This indicates that detection limit was improved through a grouping effect (compare to 1.8° for a single disc). The violet triplet was last detected when each disc subtended 4.1° and not detected at 2.5°. Thus the detectability of the violet triplet was limited by the detection limit of its elements. Since yellow targets have L-receptor contrast while violet ones do not, our results indicate that processing of grouped targets by the achromatic system differs from that by chromatic one.

[1]Giurfa et al. (1996) J. Comp. Physiol. 178: 699-709
[2]Hempel et al. (2001) J. Comp. Physiol. 187: 215-224
[3]Spaethe & Chittka (2001) Int. Conf. of Invertebr. Vis.: 240

474 Colour evaluation in concentric patterns by bees: Biological learning or sensorial constraint?

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During foraging bees must learn and decide among differently coloured displays of flowers; these are often concentric patterns. How do the bees memorise and recognise such patterns? In a previous study we tested the discrimination of two-colour concentric patterns. Bees were able to resolve pattern elements and to discriminate them from single-coloured discs and from the reciprocal pattern[1]. However, discrimination performance was affected by pattern-specific generalization: a pattern consisting of an orange centre and blue ring was not discriminated from an orange disc, whilst using the reversal arrangement, discrimination was possible. Thus bees discarded the information provided by the blue-coloured ring and paid more attention to the orange colour. Here we present experimental work testing whether chromatic or spatial cues influence the colour selectivity in concentric patterns.

Forager bees were trained in a Y-maze to a two-colour concentric pattern where both colours covered the same area. To compose the patterns, colour pairs were chosen such that they were distinguishable for bees and had similar L-receptor (achromatic) contrast in order to avoid using the latter. Stimuli subtended 30° of visual angle. In an unrewarded test single-coloured discs of both pattern colours were presented and the bee's choices recorded.

For seven out of eight colour pairs tested, bees paid more attention to one of the pattern colours. In four colour pairs, one colour contrasted more strongly with the background

than the other. Here bees consistently preferred the colour presenting higher chromatic contrast, irrespective of whether it was located in the ring or in the central element of the previously trained pattern. In the other four colour pairs, chromatic contrast to background did not vary between both colours. Bees performed differently: either none of the colours was preferred or the colour of the central pattern element was preferred. After training to a blue-green pattern, the blue colour was consistently preferred in both colour arrangements of the training pattern. Thus, the bees' attention for one colour was hue-dependent. The bees' performance in this experiment could not be correlated to the chromatic distance between pattern colours.

Bees acquire long-lasting memories for colours[2]. To test whether the observed differential evaluation of pattern colour elements was influenced by the bees' previous foraging experience, we raised and tested colour-naïve forager bees. Since bees are known to have innate colour preferences, it was necessary to determine them for our test colours. To this aim, bees were pre-trained to forage on a vertical grey disc using grey papers of different intensities. After they learned to find a reward all over the disc, an unrewarded test presenting coloured discs excluding L-receptor contrast differences was performed. The results were in agreement with previous findings[3]. We selected three colour pairs from those which previously revealed the different choice strategies. In general, the choices made by the unexperienced bees matched those of the experienced bees, although in some cases an influence of innate colour preferences and of spatial cues must be assumed.

The results indicate a sensory-biased evaluation of pattern elements rather than an influence of colour and pattern memories established during foraging life.

[1]Hempel et al. (2002) J.Comp.Physiol.A 188: 503-512
[2]Menzel (1999) J.Comp.Physiol.A 185: 323-340
[3]Giurfa et al. (1995) J.Comp.Physiol.A 177 : 247-259

Spatial distribution of colour can affect concentric pattern recognition in honeybees 475

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Bees' discrimination of simple coloured patterns can be influenced by colour generalization, which is mediated by chromatic cues[1]. Although the bee chromatic visual system has poor spatial resolution, concentric patterns were discriminated from single-coloured discs and from the reciprocal pattern on the basis of chromatic cues. Do however bees discriminate spatial variations in such patterns?

We trained free-flying bees to two-coloured concentric patterns presented in a Y-maze. Three types of patterns were trained: a dot pattern (6% central area), an equal-area pattern (50% central area) and a ring pattern (77% central area). Two additional groups of bees were trained to a single-coloured disc (yellow or cyan). The yellow and cyan colours were combined reciprocally in the patterns. Both presented the same L-receptor (achromatic) contrast, in order to guarantee that chromatic cues were used exclusively. During unrewarded tests, bees were confronted with different patterns: the training pattern and/or patterns with varying degrees of similarity .

Bees were able to discriminate patterns from each other and from single-coloured discs. Exceptions were the ring pattern and the equal-area pattern with yellow rings when bees were trained previously to the yellow disc or to the ring pattern with a yellow ring. Here they showed a random-choice distribution. Also, bees trained to the ring pattern with a blue ring (e.g. yellow covered a larger area) did not discriminate between the training pattern and the dot pattern with the wide yellow ring or the training pattern and the yellow disc. Since discrimination was performed successfully in the reciprocal tests, we conclude that this generalization effect is related to the spatial distribution of the yellow colour, which exhibited higher chromatic contrast.

Testing a yellow against a cyan disc, bees paid more attention to the colour which covered the larger area in the respective training pattern. However, after being trained to the ring pattern with a yellow ring, bees chose yellow and cyan randomly, even though cyan covered a larger area in the training pattern. In the test, bees trained to the equal-size patterns selected the yellow colour more often.

In order to assess the similarity of patterns as perceived by the bees, they were trained to the equal-area pattern and then presented with the dot and ring pattern of the same colour combination as the training pattern during the test. The result showed that the bees' similarity judgement was affected by the relative area content of the yellow colour.

In general, bees could resolve different concentric patterns through chromatic cues. Their discrimination performance, however, was impaired by specific pairings of chromatic and spatial cues. Combining larger area content and colour with higher chromatic contrast led in some cases to generalization, and thus to less accurate pattern recognition.

[1]Hempel et al. (2002) J.Comp.Physiol.A 188: 503-512

476 The role of L-receptor contrast in detection and discrimination of large-sized targets by honeybees

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Detection and discrimination of coloured targets by honeybees is mediated by chromatic and an achromatic visual pathways. While chromatic vision is mediated by three types of receptors, achromatic one is mediated by L-receptor alone[1]. At large visual angles stimuli are detected and discriminated only on the basis of their chromatic properties - L-receptor contrast has been shown to be not involved. In a recent work, the Receptor Noise Limited model of bee colour vision predicted correctly the detection of targets with high L-receptor contrast by chromatic pathway alone, however the use of the L-receptor contrast cue could not be ruled out[2].

Here we tested the detectability of four coloured targets which all either lacked L-receptor contrast or presented the same amount of high L-receptor contrast. Free-flying honeybees were trained in a Y-maze to detect a target subtending 30° of visual angle in

one of the arms during 30 visits. The four colours presented high, medium or low chromatic contrast to the background but no L-receptor contrast. By exchanging the background the L-receptor contrast was introduced for the same coloured targets.

The correct-choice-rate yielded a positive correlation with the magnitude of chromatic contrast (Spearman Rank Correlation, $p<0.001$). Detectability was neither improved by adding L-receptor contrast (χ^2, all colours NS) nor influenced by the speed of colour learning. An achromatic control target having the same L-receptor contrast as that of coloured ones was detectable for the bees (Binomial test, $p<0.05$), but performance was poor - the correct choice rate was similar to that of a coloured target having the lowest chromatic contrast. The results can be explained by a model assuming a linear relationship between contrast strength and detection output through the chromatic system without interaction with the L-receptor mediated system. This supports the hypothesis that at large visual angles only chromatic contrast is recruited for target detection.

In a second experiment we investigated the discriminability of two coloured targets (30°) of identical chromatic properties to the bee eye but with a high difference in L-receptor contrast. We used blue and brownish stimuli pairs. Bees were trained to the rewarded colour alone until reaching 80% performance level. Afterwards a 30-trial-discrimination training was performed for each bee. Both colour pairs were distinguishable for bees (Binomial test, blue: $n=120$, $p<0.001$, brownish: $n=210$, $p<0.05$). The use of a difference in chromatic contrast to background is unlikely (control blue: $n=60$, NS). We suggest that discrimination was possible due to the higher L-receptor contrast difference compared to that of previous studies (0.5-0.7 vs. 0.3 log units). It is a first direct evidence for a predicted contribution of a quite insensitive achromatic mechanism to the bee's colour vision system[3].

[1]Giurfa et al. (1996) J. Comp. Physiol. 178: 699-709
[2]Hempel et al. (2000) J. Exp. Biol. 203: 3289-3298
[3]Brandt & Vorobyev (1997) Vis. Res. 37 : 425-439

Chasing behaviour of the blowfly *Lucilia*: A smooth pursuit tracking system generates saccades **477**

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Primates, including humans, were long believed to be the only animals having an ability called smooth pursuit, i.e. continuous eye movements that follow a chosen object. Smooth pursuit is interrupted by catch-up saccades, if target motion is too rapid. However, male blowflies reveal basically the same viewing strategy when following a conspecific in the context of mating behaviour, i.e. smooth pursuit interrupted by saccadic tracking. Interestingly, blowflies are able to follow targets, even when these move one order of magnitude faster than those targets humans are able to track. Whereas in primates smooth pursuit and saccadic tracking is assumed to be mediated by separate control systems, we present evidence that, at least in blowflies, both types of following responses can be produced by the same control system.

To unravel the control system mediating pursuit behaviour of blowflies, we presented a black moving sphere instead of a real fly as target in behavioural experiments. By precisely controlling the movements of the target on a circular track, we were able to unravel the major constituents of the control system underlying smooth tracking in blowflies (Boeddeker et al. 2003 Proc R Soc Lond B 270: 393-399). As was tested by model simulations pursuit can be explained on the basis of simple phenomenological model: The retinal size of the target controls the fly's forward speed and, thus, indirectly its distance to the target. A smooth pursuit system uses the retinal position of the target to determine the fly's flight direction. Lowpass filters implement neuronal processing time. Treating the model fly as a point mass, its kinematics is modelled by Euler integration to mimic the effects of translatory inertia and air friction. Despite its simplicity, the model shows similar behaviour as real flies pursuing a smoothly moving target.

When chasing real flies, male flies change their flight direction frequently by body saccades. By model simulations we show that, without modifying the structure of the smooth pursuit model, saccadic changes of the longitudinal body axis emerge. This performance relies (1) on the characteristic relation between retinal target position and the induced turning amplitude and (2) on implementation of inertia and different time constants of lowpass filters for yaw velocity and forward velocity. Although we did not implement any saccade generating mechanism in the model fly, it can track realistically moving targets in a similar manner as do real flies. Thus, the dichotomy in the phenomenology of chasing behaviour – smooth pursuit and saccadic tracking – does not necessarily require two distinct control systems.

478 Population coding in the visual system of the blowfly: An experimental and modeling approach

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In several sensory systems information is represented by the joint activities of populations of neurons where each single neuron is tuned to a slightly different aspect of the stimulus. Theoretical studies and model simulations based on single-unit recordings obtained in a variety of systems demonstrate that the accuracy with which stimuli are encoded depends on (i) the number of cells in the population, (ii) the width and form of the tuning curves, and (iii) the variability of neuronal responses as well as the degree to which the responses of different neurons in the population are correlated (e.g. Pouget et al. 1999, Abbott and Dayan 1999).

In the fly visual system a subgroup of 20 individually identifiable motion-sensitive interneurons offers a well described biological system to investigate population coding. The sophisticated receptive field organization of these so-called VS-cells (vertical system) indicates that each cell senses a optic flow pattern induced when the animal rotates around a particular horizontal aligned body axis (Krapp and Hengstenberg 1996). From experiments on a tangential neuron, which receives input from VS-cells we infer that VS-cells have broadly overlapping tuning curves. There is also evidence from si-

multaneous double recordings that the activities of tangential neurons correlate due to common inputs (Warzecha et al. 1998), where the degree of correlated activity may depend on the degree of overlap of their receptive fields.

In electrophysiological experiments we investigate the response characteristics of VS-cells to global visual motion patterns as generated on the eyes during rotations of the animal about different horizontal body axes. Relevant response characteristics are the preferred axis of rotation, the form of the tuning curves, the amount of noise in the neuronal responses and the extent of correlated activity in neighboring cells. Using a Maximum Likelihood estimator the accuracy is determined with which a certain self-rotation of the animal can be detected on the basis of the neuronal population response. In model simulations of the system we test how this accuracy is affected by manipulations of the population activity, e.g. the elimination of single cells or by shifting the tuning curves to different preferred axes of rotation.

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In vivo manipulation of Ca^{2+} regulation in visual motion-sensitive neurons of the fly by flash photolysis of caged Ca^{2+}-chelators

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In visual motion-sensitive neurons of the fly, the involvement of intracellular Ca^{2+} in synaptic transmission and adaptation has been studied [1,2]. So far, conclusions about the potential role of Ca^{2+} in activity-dependent adaptation have been drawn based on correlations between Ca^{2+} concentration changes and the electrical activity of the neurons. Our aim is now to find clear causal links between Ca^{2+} and adaptation mechanisms. Moreover, we want to analyze the role of Ca^{2+} in synaptic functioning by actively interfering with the intracellular Ca^{2+} concentration.

We filled single neurons with one of the caged Ca^{2+} chelators DMNP or NP-EGTA and managed to set free Ca^{2+} in the cytosol by delivering UV light from a Xenon flash lamp via a quartz light guide directed to the neuronal arborizations. By flash photolysis, the cytosolic free Ca^{2+} concentration could be spatially homogeneously raised in a step-like manner to concentrations comparable to those which are typically measured when stimulating the neurons by visual motion. After photolysis, the Ca^{2+} concentration falls back to baseline levels with a faster timecourse in fine neurites as compared to the thick branches. This suggests, that Ca^{2+} clearance is influenced by the surface-to-volume ratio of the neurite, indicating a major contribution of extrusion mechanisms located in the outer neuronal membrane.

We are beginning to employ flash photolysis of caged Ca^{2+} in order to study the dependence of postsynaptic activity on presynaptic Ca^{2+} at synapses between identified motion-sensitive neurons. One type of synapse between a graded potential presynaptic neuron and a spiking postsynaptic neuron, was found to operate fairly linearly when presynaptic neurons are driven by sensory stimuli [2]. In a second project, we will investigate, whether the manipulation of the dendritic Ca^{2+} concentration has an impact on neuronal excitability, as would be predicted by the hypothesis of Ca^{2+}-dependent adaptation [1].

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480 Does the signal form of blowfly motion-sensitive neurons depend on recording quality?

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As in other peripheral sensory systems, different types of motion-sensitive tangential cells (TCs) in the third visual neuropile of the blowfly may respond in their output terminal either by sequences of action potentials or by graded shifts in their membrane potential. However, there are also TCs that show a mixed response mode, i.e. graded membrane potential changes that are superimposed by rapid spike-like depolarisations. The amplitude of these so-called spikelets is very variable: In some preparations they are hardly visible at all. In others, however, they may reach peak amplitudes of 50 mV and more. In preparations, that do not show pronounced spikelets in their "normal" state, large-amplitude spikelets may emerge when the cell is artificially hyperpolarised by intracellular current injection (e.g. [1,2]). There is pharmacological evidence that spikelets are based on voltage-sensitive sodium currents [3].

The variability in the spikelet amplitude raises the question as to the functional significance of these rapid membrane potential changes. Is visual motion information conveyed mainly by the graded response component, or by the spikelets or by both types of membrane potential changes? This question is difficult to answer, because the spikelet amplitude is very prone to changes in membrane polarisation. Since it is well possible, that TCs are slightly depolarised as a consequence of penetrating them with an intracellular microelectrode and of the resulting leak currents, it is hard to infer the natural signalling mode in the undisturbed cell. In other words, it is not clear whether the variability found in the signalling mode of these TCs is the consequence of genuine variability between different preparations and/or different cell types or whether it simply reflects differences in the recording quality.

To answer this question we relate the input resistance of TCs under their resting conditions (i.e. without motion stimulation) as well as during preferred and null direction motion to the amplitude to the graded response component as well as to the size of the spikelets. If the size of the spikelets were systematically correlated with the cell's input resistance, recording quality is likely to be a major determinant of the cell's signalling mode. On the basis of these experiments we plan to derive objective standards for good recording quality in fly TCs.

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Impact of photon-noise on the reliability of a motion sensitive neuron in the visual system of the blowfly *Lucilia* 481

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The precision with which visual stimuli can be encoded by neurons is constrained by several noise sources such as photon-noise, synaptic noise or the stochastic nature of ion channels. Recently, response variability of the fly's H1-cell, a motion-sensitive visual interneuron, was attributed to photon-noise[1,2]. Hence, the fly's visual system is thought to be a perfect encoder limited by the physical limitations of the input signal. On the other hand, it was concluded - at least for the light adapted eye - that noise sources inherent in synaptic transmission between photoreceptors and 2nd-order neurons significantly affect the reliability with which visual information is signalled to higher-order processing stages[3,4]. Here we address whether photon-noise limits the reliability of responses of H1 to visual motion stimuli.

In an approach adapted from psychophysics the motion stimuli were superimposed by external luminance noise of increasing strength. We determined the lowest noise level which significantly influences the cell's responses. An effect is regarded as significant when responses elicited by a motion stimulus superimposed with a specific noise sequence can be discriminated from those responses evoked by the same motion stimulus superimposed by a different noise sequence with the same statistical properties. We quantified the discriminability with an ideal observer paradigm using a number of temporal resolutions ranging from fine resolutions looking at the precise temporal structure of the responses up to the mean spike-rate ignoring the time-structure. Since the noise level found to influence the responses significantly is much larger than the estimate of photon-noise under the experimental conditions, our results indicate that H1 response reliability is more likely to be limited by internal sources of variability than by photon-noise.

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482 High resolution imaging of presynaptic calcium with two-photon-microscopy

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In many cell types calcium plays an important role in mediating cellular processes. In neurons different aspects of information processing rely on changes in intracellular calcium concentration.

A prominent function of calicum is to regulate the output of chemical synapses. Since many calicum-dependent processes, such as the opening of voltage-dependent calcium-channels or the cooperative binding of calcium molecules to the receptor that triggers transmitter-release, are highly nonlinear, it is not easily possible to infer on the overall synaptic performance under natural operating conditions.

To overcome this problem we investigate synaptic transmission under *in vivo* conditions in a pair of synaptically connected motion-sensitive tangential cells of the blowfly. The presynaptic neuron signals motion information by graded changes in its memrbane potential that may be superimposed by spike-like depolarizations. The almost planar aborizations of the tangential cells make them well suited for calcium imaging. In previous experiments , presynaptic calcium concentration changes were measured during visual motion stimulation by applying conventional fluorescence microcopy to the *in vivo* preparation. The relationship between presynaptic calcium concentration changes, depolarizations and postsynaptic spike-rate was found to be almost linear over the entire range of activity levels that can be evoked by sensory stimulation.

To further increase the temporal and spatial resolution of imaging calcium concentration changes of different branches in the synaptic area, we applied two-photon-microscopy to our *in vivo* preparation. The time-courses of motion-induced presynaptic calcium accumulations could be visualized by line-scans using two-photon-exciation. Here, a difference in time-course was found in neurites of different diameter: The decline of calcium concentration after cessation of visual motion stimulation was faster in fine presynaptic arborizations compared to the main branch of the presynaptic terminal. An explanation for the dependency of calcium decline on branch diameter is that calcium clearance is limited by transport mechanisms located in the outer cell membrane. So the velocity of calcium decline is dependent of the neurite`s surface-to-volume ratio.

In further studies it is planned to perform line-scan-measurements of different branches in the synaptic area along more than one line simultaneously by multifocal-two-photon-microscopy.

The Visual Receptive Field of a Fly Neck Motor Neuron 483

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Sensory information is processed through multiple stages in the nervous system before an appropriate motor output is produced. Understanding the sensory-motor circuits involved requires a description of how the representation of sensory information changes across the different processing stages. Here we present the first results of a project designed to compare the visual response properties of visual interneurons in the fly lobula plate with those of their target motor neurons.

Many animals, including humans, estimate their own movement through space by analysing the resulting panoramic visual motion, optic flow. Flies utilise optic flow to guide various behaviours, including gaze stabilising head movements. Some motion sensitive tangential cells within the fly lobula plate are well adapted for representing rotational optic flow (Krapp, Intern Rev Neurobiol 44:93-120, 2000). Theoretically, just three cells could represent rotational optic flow, each signalling a rotation about one of three Cartesian axes. This orthogonal representation is not seen in the lobula plate. Approximately 13 output tangential cells are involved, each one tuned to the optic flow resulting from rotation about a different, non-orthogonal axis. How is the lobula plate's highly non-orthogonal representation of optic flow used to generate appropriate motor outputs? To answer this question we are studying the visual response properties of neck motor neurons. These motor neurons receive direct synaptic inputs from the lobula plate and drive the neck muscles responsible for gaze-stabilising head movements (Milde et al. J Comp Physiol A 160:225-238 1987, Strausfeld et al. J Comp Physiol A 160:205:224 1987).

Extracellular recordings are made from neck motor neurons within the cervical nerve while presenting local motion stimuli in different parts of the visual field (Krapp & Hengstenberg, Vision Res 37:225-234, 1997). Using this method, maps are created, revealing how the neurons' preferred direction of motion and motion sensitivity vary across their receptive fields. We find that one neuron within the cervical nerve has a receptive field organisation ideally suited to optic flow resulting from an intermediate pitch/roll movement of the fly. The results demonstrate that optic flow information reaches neck motor neurons in a form similar to that seen in the lobula plate's tangential cells. Thus, part of the sensory-motor transformation occurs at the level of the lobula plate. The presence of a neuron representing information about an intermediate pitch/roll self-movement argues against the neck motor system containing neurons tuned only to pure pitch, roll and yaw. A reasonable hypothesis is that the neuron's preferred axis of rotation is aligned with the pulling plane of the muscle it innervates.

484 Adaptation to lifestyle in the visual system of solitarious and gregarious locusts

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Desert locusts *Schistocerca gregaria* exist in a range of forms between two morphologically and behaviourally distinct extremes, the solitarious and gregarious phases. One marked difference in behaviour is that solitarious animals fly individually whereas gregarious locusts fly in dense swarms. We show that there are changes in identified visual neurones that can be related to this change in behaviour.

Touching hairs on the hind legs of a solitarious locust can within 4h transform that animal's behaviour so that it acts gregariously. We can also drive this change by electrically stimulating a leg sensory nerve.

We have analysed the responses of a visual interneurone, the descending contralateral motion detector (DCMD), and the properties of its output connections onto a leg motor neurone, the fast extensor tibiae (FETi) that is important in escape jumping. DCMD responds maximally to the sight of an object looming towards the locust on a collision course.

The responses of DCMD were tested using simulated looming objects of various sizes and velocities, presented at 1 min intervals. DCMD in gregarious locusts showed little habituation to repeated stimulation, but in solitarious locusts it showed a pronounced habituation to 30% of the value recorded in gregarious locusts.

A spike in DCMD elicited a monosynaptic excitatory postsynaptic potential in the metathoracic FETi, the amplitude of which was approximately 150% larger in solitarious locusts than in gregarious locusts. Thus although fewer action potentials were elicited in DCMD by visual stimuli in solitarious locusts, each carried a far greater weight at this output synapse onto FETi. This tunes the visual pathway in solitarious animals so that it is maximally sensitive to infrequent visual stimuli (which is likely to be the normal situation for these animals living singly in large expanses of desert). The same visual pathway of gregarious locusts is tuned by virtue of its resistance to habituation but weaker synaptic strength so that it continues to function effectively even when the visual environment surrounding the locust contains moving objects. This may help gregarious locusts avoid collisions with conspecifics during swarming, or it may serve to maintain the sensitivity of the escape circuit to the sight of an approaching predator even when surrounded by many other locusts.

This work was supported by the BBSRC.

Localization and characterization of an extraretinal photoreceptor in the brain, retrocerebral complex, and frontal ganglion of sphingid moths (Lepidoptera: Sphingidae) 485

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We have detected extraretinal photoreceptors in the brain, the retrocerebral complex, and the frontal ganglion of four hawkmoth species (*Acherontia atropos*, *Agrius convolvuli*, *Hippotion celerio* and *Manduca sexta*), using Western Blot analysis, immunocytochemistry and immunoelectron microscopy. A specific antiserum of *Papilio glaucus* (*PglRh4*) exposed a widespread distribution of an `exo-opsin´ in 200-300 neurons of the optic lobes, the supra- and suboesophageal ganglion, and the antennal lobes. All major neuropil regions are innervated by these neurons. In addition, several perikarya were immunoreactive in the frontal ganglion, the corpora cardiaca, and the corpora allata. Immunoelectron microscopy showed that this type of extraretinal opsin is located in myeloid-like bodies in these neurons.

A further specific antibody revealed melatonin in or close to the opsin containing perikarya. This finding suggests a role for the entrainment of a photoperiodic rhythm in sphingid moths, as this biogenic amine is known to have a great seasonal influence on reproduction, development, longevity and behaviour in animals. Thus, these photosensitive neurons may play a similarly important role as the photoperiodic counter and neuroendocrine pathway of the pinealocytes in nonmammalian vertebrates.

3D representation of the landing approach of *Libellula depressa* in a study of navigation mechanisms in natural surroundings 486

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The dragonfly *Libellula depressa* was chosen for video studies of the landing approach in natural surroundings.

Near a pond, an artificial perch was provided for the dragonflies. Two analogue video cameras were positioned 2 m from the perch, at an angle of 90° to one another, in order to record the last 50 cm of the landing approaches. A synchronisation cable between the two cameras permitted precise synchronisation of the frames. A frame coder connected in series with the video recorder provided both camera signals with a time stamp. In conjunction with a computer program, this technique permitted a three-dimensional analysis of each individual frame. The last 20 cm of the landing approaches were studied using only one camera; in this case only landing approaches where the dragonfly was moving in the picture plane were analysed.

It was found that the dragonfly does not approach the perch directly, in a straight line. In the last 50 cm of the landing approach, the dragonfly describes a curved path comprised of forward motion combined with translational horizontal and vertical motion. The dragonfly usually flies in a small arc around the perch, during which the longitudinal axis of the body is oriented not in the direction of flight, but in the direction of the landing target. During this manoeuvre the insect attempts to keep the landing target always within the field of view of the ventral region of the eye. This is accomplished not only via the above-described flight pattern, but also by means of vertical tilting of the body axis. Results so far indicate that during the whole landing approach the head forms a straight line with the body axis, however during lateral tilting motions the head maintains its horizontal alignment in space.

At a distance of 11 cm from the target, the dragonfly begins to open out its hindmost pair of legs. The angle of opening exhibits an exponential increase until landing occurs. It was also found that during the final centimetres of the landing approach, the target is maintained within the ventral field of view solely by means of vertical tilting of the body.

Relative motion of retinal images could aid in the detection and fixation of landing objects; here the insect makes use of the different angular velocities of objects at different distances. The translational horizontal and vertical motions could aid in the determination of relative distances. As soon as the dragonfly begins to navigate in a straight line towards the target during the final centimetres of the approach, image expansion in the ventral field of view could aid in absolute distance determination. During the final 20 cm of the landing approach, the image of the landing target becomes exponentially more ventral; the more ventral the image, the more accurate binocular input.

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487 Neurons at different levels of the locust optic lobe detect looming objects.

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Locusts must recognise obstacles occurring in their path and the approach of a predator before they can react with escape, steering manoeuvres or a hiding response. Therefore, the perception of looming objects is an important feature to be achieved by the visual system of these animals. Most studies on perception and processing of looming stimuli and the initiation of escape behaviours in locusts have focussed on the lobula giant movement detector (LGMD) and the descending contralateral movement detector (DCMD, review by Rind 2002). They react mainly to looming stimuli which appear very fast somewhere in the visual field and also to those appearing on direct collision course. This system is not able to control e.g. the hiding response of sitting locusts, a behaviour that is triggered by dark objects expanding slowly to over 10o in the visual field and the turn angle of the movement depends on the stimulus location in the visual field (Hassenstein and Hustert, 1999).

Since hiding responses include spatial localisation of a stimulus we searched for interneurons that could contribute to the perception and location of looming stimuli at different levels of the optic lobes and in the brain of L. migratoria. Appropriate looming stimuli presented at adjacent sites in the lateral visual field revealed neurons at different levels of the visual pathway responsive to expanding stimuli, which might be involved in the perception of looming. Some neurons react nearly exclusively to stimuli in a narrow region of the visual field. They are located in the lamina or in the medulla and project to the lobula or the protocerebrum. Similar neurons, distributed in more distant regions of the visual field are to be expected.

A variety of potential parameters for looming detection we investigated at the level of single neuron responses. Changing distance of the eye to an object producing a retinal image might be coded by features as decreasing overall luminance, and/or increasing image size, and altering perimeter of an image. The stimuli we used represent all or only a part of the information of a looming object: an expanding disk which imitates all parameters of looming, an expanding ring (annulus) which depicts only increase of perimeter length, and we inverted the brightness of the circle to test dependence of the reaction on the luminance. Different neurons depicted various parameters of looming. Some reacted especially on the brightness and/or on the darkness, others responded to the perimeter or on the visual angle taken by the visual stimulus. For some of the protocerebral neurons we could not clearly delimit the parameters. They are possibly involved in later stages of processing, dealing not only with looming detection.

The perception of looming objects is based on a complex network reflecting the retinotopic construction of the visual system with different stages of processing. Apparently there are parallel pathways for processing looming features of a visual stimulus. Evidently, specific behavioural responses cannot rely solely on the LGMD/DCMD-system.

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Identification of cells showing cyclical expression of Na^+/K^+-ATPase in the visual system of *Drosophila melanogaster* 488

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The Na^+/K^+ pump is a major plasmalemma transport ATPase that maintains the Na^+ and K^+ gradients essential for neuronal and glial cell function. Its expression may provide a useful tool to study temporal changes in the patterns of activity involved in signal transmission. In the fly's visual system, for example, such dynamic events could under-

lie circadian changes controlled both by direct light stimuli and internal signals from a circadian clock (Pyza E & Meinertzhagen I.A., 1995: J. Neurosci. 15:407), which could be visualised as Na^+/K^+-ATPase activity.

To visualize neurons and glia in the visual system of living *Drosophila melanogaster* we have used a transgenic line expressing green fluorescent protein (GFP) under control of a tissue-specific transcriptional regulatory element for the Na^+/K^+-ATPase β subunit gene, *Nervana2* (Xu P, et al, 1999: Gene 236:303). GFP fluorescence in this line is sufficient to allow Na^+/K^+ pump activity to be imaged directly through the head cuticle. We have measured this activity at different times of the day and night (ZT2-ZT10 and ZT14-ZT22) by measuring the intensity of GFP fluorescence through the posterior occiput. Digital images from flies previously entrained to day/night (LD 12:12), night/day (DL 12:12) or constant darkness (DD) conditions were converted to grey-scale in Adobe Photoshop and the Mean Grey Value of the entire head was quantified using NIH Image software.

There were daily changes in GFP expression with significantly brighter mean fluorescence during the day ($t = 4.15$, $p < 0.001$). Although the intensity of fluorescence was lower under DD, the rhythm in these changes was maintained ($t= 6.97$, $p < 0.001$), indicating its endogenous origin. To identify and characterise cells that cyclically express GFP, Vibratome and cryostat sections of adult brains were processed for immunocytochemistry with the following antibodies: a photoreceptor-specific Mab 24B10 against Chaoptin (Hybridoma Bank); anti-neuronal Mab 22C10 (Hybridoma Bank); glial cell-specific anti-Repo; anti-Ebony polyclonal and Mab 236 labelling epithelial glia; and a polyclonal against synthetic pigment-dispersing factor (PDF). Immunolabelling showed that neither photoreceptors nor other neurons expressed GFP reporter in the retina, lamina or medulla. Cells showing GFP expression were identified as glia based on their positions and the co-expression of Repo protein (Halter DA, et al, 1995: Development 121:317). There were also Repo-positive glia which did not express GFP. These were localized in the lamina and expressed immunoreactivity to both the Ebony and Mab 236 antibodies against epithelial glia. Those that showed GFP expression were mostly located in the medulla next to processes of neurons expressing PDF.

These results are compatible with the possibility that these glial subtypes may play an active role in regulating temporal changes in neurons through circadian fluctuations in Na^+/K^+-ATPase expression.

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Pigment-dispersing hormone (PDH)-immunoreactive neurons form direct coupling pathways between the bilaterally symmetric circadian pacemakers of the cockroach *Leucophaea maderae* 489

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The circadian periodicity of the locomotor behaviour of the cockroach *Leucophaea maderae* is driven by two bilaterally paired and mutually coupled pacemakers, which reside in the brains optic lobes (Page 1982, Science 216:73-75). Transplantation studies located the circadian pacemaker in the accessory medulla (AMe), a small neuropil of the optic lobe (Reischig and Stengl, J. Exp. Biol. in press). The AMe is densly innervated by anterior and posterior pigment-dispersing hormone immunoreactive (PDH-ir) medulla neurons (PDHMe), which are circadian pacemaker candidates in *Drosophila melanogaster* as well as in the cockroach (Helfrich-Foerster et al. 1998, Chronobiol Int 15:597-564). The anterior PDHMe somata can be devided into three subgroups according to morphological criteria: the small, medium-sized, and large PDHMe. We wanted to know whether a subpopulation of these cells serve as circadian coupling pathways, directly connecting both pacemakers centers (Reischig and Stengl 2002, J Comp Neurol. 443:388-400).

In search for the coupling pathways between both clocks, we injected rhodamine-labelled dextran as neuronal tracer into one AMe and combined it with PDH-immunocytochemistry. The preparations were examined with confocal laser scan microscopy. Double-labelled fibers in the AMe contralaterally to the injection side showed that PDH-ir fibers directly connect both AMae. The double-labelled fibers arborize in the anterior neuropil, in the shell neuropil, and, to a lesser extend, in the internodular neuropil of the contralateral AMe. Three PDHMe of each optic lobe, which all belong to the anterior PDHMe, but not to the posterior PDHMe directly connect both AMae. One of them reaches the contralateral AMe via the posterior optic commissure, the other two via the anterior optic commissure. Two of the anterior PDHMe projecting to the contralateral AMe belong to the large, and one to the medium-sized anterior PDHMe. Thus, we could show that indeed a subset of the PDH-ir neurons of the medulla directly connect both AMae and therefore, most likely contribute to circadian coupling of the two circadian pacemaker centers.

In the central brain, all except one of the typical projection areas, namely the ventrolateral protocerebrum, are innervated by anterior PDHMe of the ipsi- as well as of the contralateral optic lobe. Additionally, a fourth anterior PDHMe soma projects to the contralateral central brain via the anterior optic commissure, but does not reach the contralateral optic lobe. This indicates, that not only the pacemaker centers are mutually synchronized, but that additionally most output targets of the clocks are affected by both pacemakers. Therefore, our data suggest that anterior PDHMe neurons play multiple roles in generating circadian rhythms, delivering output of timing information, and performing mutual pacemaker coupling in *L. maderae*. [Supported by DFG grant STE 531/7-2,7-3 and by Human Science Frontiers]

490 Immunocytochemical localization of the presumptive clock protein PERIOD in the cockroach *Leucophaea maderae*

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In the cockroach *Leucophaea maderae* the circadian rhythm of locomotor activity is controlled by bilaterally symmetric, apparently directly coupled, circadian pacemakers in the optic lobes. Transplantation experiments showed that the accessory medulla (aMe), which is located at the ventromedial edge of the medulla in the optic lobes, is this circadian clock (Reischig and Stengl, J. Exp. Biol. 2003 submitted). In the cockroach as well as in *Drosophila melanogaster*, the accessory medulla is densely innervated by pigment-dispersing hormone-immunoreactive (PDH-ir) neurons, which are circadian pacemaker candidates in different insect species. In *D. melanogaster* PDH-ir neurons express the circadian clock molecule PERIOD (PER; Helfrich-Förster, 1996, PNAS 92: 612). PER is part of a molecular feedback loop that underlies a circadian rhythm in PER protein and mRNA abundance, with the PER-protein peak occuring at ZT 21 at the late subjective night, and the minimum occuring during the middle of the day.

In this study we investigated immunocytochemically with a polyclonal anti-*Drosophila* PER antibody (provided by M. Young and L. Saez), whether or not PER-like proteins occur in the cockroach brain, and whether they show cycling in protein abundance. Staining in the optic lobes and in the brain could be found in the cytoplasm and in the nuclei. In the optic lobes cytoplasmic PER-immunoreactivity was observed next to the accessory medulla, to the lobula, and in the lobula-organ. Cytoplasmic staining in the brain was found close to the antennal lobes, in the pars intercerebralis, and sporadically also in the lateral protocerebrum and pars lateralis. Staining of the nuclei occured widespread in all areas of the brain.

In the optic lobes as well as in the brain a circadian rhythm of staining intensity was observed with a maximum at ZT 15 and a minimum at ZT 11. The difference between the maximum and the minimum was statistically significant in the antennal lobes and the pars intercerebralis. Counts of the cells with stained cytoplasm revealed a mean number of 2 cells next to the accessory medulla, 7 to the lobula, 140 to the antennal lobes, and 24 to the pars intercerebralis. Currently, we test for specificity of the staining with PER-protein from *Drosophila* (provided by L. Saez and M. Young). Crucial for the generation of species-specific antibodies is the cloning of the *L. maderae-per*-gene, which is currently in preparation. [Supported by DFG STE 531/7-3 and by Human Science Frontier]

Behavioral evidence of polarization vision in the locust *Schistocerca gregaria* 491

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Many insects are able to detect the polarization pattern of the blue sky and use this capability for spatial orientation (Wehner R 1984, Annu. Rev. Entomol. 29, 277-298; Wehner R, Michel B and Antonsen P 1996, J. Exp. Biol. 199, 129-140). Ommatidia specialized to detect skylight polarization are confined to a small dorsal rim area (DRA) of the compound eye. The desert locust *Schistocerca gregaria* has a particularly prominent DRA (Homberg U and Paech A 2002, Arthropod Struct. Dev. 30, 271-180), and polarization sensitive neurons have been identified in several brain areas (Vitzthum H, Muller M and Homberg U 2002, J. Neurosci. 22, 1114-1125). These results strongly suggest that desert locusts use the sky polarization pattern for navigation.

In the present study we have developed a behavioral assay to investigate polarization vision in the locust. Experiments were performed on tethered flying locusts fixed to a yaw-torque meter (Preiss R and Gewecke M 1991, J. Exp. Biol. 157, 461-481) in front of a wind tunnel. A rotating polarization filter (speed 5.2 °/s), illuminated from the back, was presented from dorsal. The following control experiments were performed: (1) the polarization filter was replaced by a diffusor (translucent drawing paper); (2) locusts were flown under the polarization pattern with the DRAs painted black; (3) as (2), but the DRA was left open and the rest of the eye was painted black; (4) the polarization filter was placed directly behind the diffusor (intensity gradient). In the test situation and in control (3), the locusts showed strong polarotaxis. In response to the rotating polarization filter the animals showed periodic changes in turning tendency every 180°. In contrast, periodic yaw-torque responses were not induced in the control situations (1), (2), and (4). E-vector orientations that did not induce a turning response, the so-called preferred and avoided e-vector, showed high individual variability and were broadly distributed from 0-180°. This study demonstrates polarotaxis in stationary flying locusts and shows that photoreceptors in the DRA of the compound eye are essential for this behavior. Supported by DFG grant HO 950-13.

Neurons of the anterior optic tubercle of the locust *Schistocerca gregaria* are sensitive to the plane of polarized light 492

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The anterior optic tubercle (AOTu) is a small neuropil in the insect brain. In the desert locust *(Schistocerca gregaria)*, the AOTu is located near the frontal surface of the brain between the calyx of the mushroom body and the antennal lobe about 200 mu m lateral to the α-lobe. As in many other insect species the AOTu of the locust is divided into an

upper and a lower subunit (Jawlowski 1963, Acta Anat, Basel 35:346-359). Recent morphological work on the AOTu of the locust showed that its two subunits are connected to topographically different subunits of the lobula, the medulla, the lateral accessory lobe and the contralateral AOTu and lobula (Homberg et al., submitted to J Comp Neurol). A population of neurons from the lower subunit projects to distinct areas of the lateral accessory lobe (the lateral triangle and the median olive), that are believed to be input regions for polarization-sensitive neurons of the central complex (Vitzthum et al. 2002, J Neurosc 22:1114-1125).

We investigated the responses of AOTu neurons to visual stimuli by means of intracellular recordings combined with Neurobiotin injections. Neuronal responses were tested in particular to linearly polarized light with rotating e-vector and light spots, positioned at different azimuths to the locust.

Most neurons with branches in the lower subunit interconnected both AOTus via the intertubercle tract and some of them had additional ramifications in the anterior lobe of the lobula. One type of neuron stayed ipsilateral and projected into the lateral triangle of the lateral accessory lobe. All neurons of the lower subunit were polarization sensitive and showed polarization opponency, i.e. the e-vector that elicited maximum spiking activity was perpendicular to the e-vector that elicited minimum spiking activity. This property is also characteristic for polarization-sensitive neurons of the central complex, which are assumed to be part of a sky compass navigation system. Overlapping branching areas between AOTu neurons and central-complex neurons, as well as their physiological similarities suggest that the lower subunit of the AOTu is part of the sky compass navigation system and provides input into the central complex.

None of the neurons recorded from the upper subunit of the AOTu was polarization sensitive, but some of them reacted to other stimuli such as stationary light or tactile stimuli. The neurons either interconnected both AOTus or projected from the AOTu to large areas in the median protocerebrum. Unlike the lower subunit of the AOTu the upper subunit cannot be assigned a functional role yet.

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493 Dissecting the Neural Network of the Fly Lobula Plate

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As a fly buzzes around its world the visual scene moves constantly across its eyes. Depending on the flight path, this elicits a particular large-field motion pattern called ‘flow field’. Since the flow-field is characteristic for the flight trajectory, it can be used to guide behavior. Large field motion information is processed in the lobula plate of the blowfly where there exist approximately 60 neurons on each side of the brain. The group of neurons that process horizontal motion form a symmetric bilateral network that is able to combine information about motion presented in front of both eyes. Here we consider a group of 16 neurons whose connections have been explicitly identified (Haag and Borst, 2001, Haag and Borst, 2002).

Each of these neurons has a large dendritic tree that receives information about ipsilateral local. There, local motion cues are spatially pooled such that each neuron produces a directionally selective response to motion presented in front of the ipsilateral eye. In addition, some of the lobula plate neurons are also sensitive to motion cues in front of the other eye. This information is carried by the spiking neurons H1, H2 and Hu that send their axons to the other side of the brain, where they synapse onto 2 of the 3 horizontal system (HS) and both centrifugal horizontal (CH) neurons. These network interactions appear to amplify the response to rotational stimuli and reduce the response to translation.

We are using laser ablation techniques and electrophysiology to dissect this circuit and determine the functional significance of the various network connections. This has begun by focusing on the input circuitry of HS and CH neurons. HS neurons are known to receive information about local motion cues directly from other visual neuropiles (medulla). On each side of the brain there are 3 HS and 2 CH neurons. The responses of HS and CH-cells to visual motion are remarkably similar. In addition it is known that these two neurons' overlapping dendritic trees are electrically coupled (Haag and Borst, 2002). We can show that CH neurons receive ipsilateral motion information only via the HS dendritic tree and not directly from local columnar elements.

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Network interactions between lobula plate tangential cells of the blowfly

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The lobula plate tangential cells of the fly visual system represent a set of about 60 large neurons per brain hemisphere that can be individually identified by its invariant anatomy and characteristic visual response properties. These neurons have large receptive fields and respond to visual motion in a directionally selective way. Some of the lobula plate tangential cells exhibit a strong selectivity for particular flow-fields. Such receptive field structures cannot arise through input from respectively oriented small-field elements alone but are additionally due to network interactions between various large-field neurons. Using dual cell recordings - either intra/extracellular or intra/intracellular - we investigated the connectivity between tangential cells within one lobula plate as well as between the lobula plates of both brain hemispheres. Amongst the horizontal cells, both HS- and CH-cells receive contralateral input from heterolateral spiking neurons H1 and H2, CH-cells additionally from Hu (Hausen, 1984; Haag, 1994; Horstmann et al., 2000). These connections form the basis for the sensitivity of both HS- and CH-cells to visual motion stimuli presented in front of the contralateral eye. In addition we found that HS- and CH-cells influence those heterolateral spiking neurons whose dendrites are located on the same side: They excite the ones with the same preferred direction (Hu) and inhibit the ones with the opposite preferred direction (H1, H2). This leads to a suppression of the activity of the contralateral HS- and CH-cells upon excitation of HS- and CH-cells on the ipsilateral side of the brain (Haag and Borst, 2001). The circuit, thus, favors

motion stimuli that lead to an inhibition on one side and to an excitation on the other side, i.e. rotational flow-fields. Besides the connectivity between horizontal cells, we also examined interactions between the horizontal and the vertical system. We found two different pathways mediating this interaction: 1) the mainly vertical sensitive VS1 synapsing onto the horizontal sensitive spiking neurons H1 and H2, and 2) the vertical sensitive VS1-VS3 cells synapsing onto the spiking V1 cell, which in turn is presynaptic to the vCH-cell of the contralateral brain hemisphere.

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495 Neural image processing by dendritic networks

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Convolution is one of the most common operations in image processing, leading to a blurring or a sharpening of the image depending on the weighting function. Many brain areas consist of topographically organized columns, which form a matrix representation of the sensory surface maintaining neighborhood relations between successive processing layers. This arrangement provides the necessary structure on which convolution can be applied. Visual interneurons in the lobula plate of the fly integrate over such columns of local motion-sensitive cells to compute large-field movement for course control. Recent evidence supports the presence of dendritic electrical coupling between two lobula plate interneurons, the HS- and the CH-cell (Haag & Borst, 2002). By this way, the topographic potential distribution in the dendrite of the HS-cell is passed onto the dendrite of the CH-cell, resulting in a blurring of the motion image in the latter (Haag & Borst, 2002). We investigated such a connectivity with existing compartmental models (Borst & Haag, 1996; Haag et al., 1997) by arranging them appropriately to form a dendritic network. Electrical synapses were modeled by linear conductances without any further dynamics.

In general, the model fitted the experimental data extremely well. It also resolved some discrepancies of previous modeling studies where the CH-cell was assumed to receive direct input from columnar elements instead of indirectly through the HS-cell (Haag et al., 1999). Most importantly, the model revealed a blurring of the dendritic potential distribution similar to the experimental data. In a simplified model consisting of two electrically coupled linear cables, we found that the blurring effect is an inherent property of such a dendritic network with the weighting function determined by the dendritic filtering properties.

Since the spatially blurred motion image in the CH-cell is passed onto a third cell via inhibitory dendro-dendritic synapses this could result in a sharpening of the signal. This enhancement of motion contrast provided by the dendritic network could then be the central element of figure-ground discrimination based on relative motion in the fly.

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Transcriptome studies in the visual system of the fruitfly 496

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The molecular organization of information processing is one of the major tasks in neurobiology. Among the different sensory modalities that are processed in different parts of the brain, visual information processing is the most important. This is not only true for man and most mammals, but also for most insects, especially those where flying is the preferred way of motion. Transduction processes take place in the sensory cells and different types of processing are performed in different parts of the retina as well as in different pars of the visual centers of the brain. In the fruitfly *Drosophila melanogaster*, visual information is transduced in the sensory cells of the complex eye. Within the retina 8 different photoreceptor cells form a single ommatidium. The photoreceptors R1-R6 make synapses within the first visual neuropile of the optic lobe, the lamina with so-called lamina-monopolar cells. The axons of the photoreceptors R7 and R8 travel through the lamina to make synapses with yet unidentified neurons of the medulla. Information is further processed in the different neuropiles of the visual system, the lamina, the medulla, and the lobula. To identify the molecules that are transcribed in either the areas of interest, we took advantage from different methods to isolate specific parts of the visual pathway. The retina as well as the first neuropile of the optic lobes, the lamina could be isolated using a modified freeze-drying method. Aceton-drying of total flies enabled very pure isolation of both, the retina and the lamina, both devoid of any contaminating tissue. On the other hand, the use of specific Gal4 lines crossed to UAS-lines expressing GFP allows to isolate specific neuronal populations. Among them are subpopulations of the lamina-monopolar-cells enabling transcriptomal analyisis of cells postisynaptic to photoreceptors.

In addition, we isolated the retinae as well as the laminae of flies lacking one photoreceptor, the R7 photoreceptor (sevenless). The retinae of these flies are normal except the lack of this photoreceptor type. In addition, it could be assumed that these flies have deficiencies in the medulla, where these photoreceptors make synapses with yet unidentified first-order visual interneurons.

We isolated the RNA from the different tissues mentioned above and prepared the hybridization probes. Differential hybridization revealed different features: The almost complete retinal transcriptome, the transcripts that are specific for the photoreceptor R7 and the transcripts of the different part of the neuropiles of the fruit flies optic lobe. Analysis of the complete set of transcription experiments should give us novel insights into the molecular organization of the visual pathway as well as into the differential transcription in the photoreceptor population.

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497 New Insight into the Landmark Orientation Behavior of Freely Walking Fruit Flies: Both Object Distance and Azimuth Position Matter

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Freely walking fruit flies (*Drosophila melanogaster*) show a strong preference for the nearest landmark (LM) when presented with several similar LMs differing only in their distance. The flies use self-induced parallax motion of the LM image on the eye to evaluate distance (Schuster et al. 2002, Curr Biol 12:1591-4). The amplitude of the parallax motion depends not only on the distance but also on the angular position of a LM image on the eye of the fly. The closer the LM the larger is the shift. The shift increases from frontal to lateral LM positions on the retina and it is greatest at 90°. In the present study we try to further elucidate the mechanisms underlying landmark choice.

Orientation behavior was measured by tracking the position and orientation of single flies on a circular platform in the center of a cylindrical display consisting of 5760 light-emitting diodes. On this display we are able to let LMs appear or disappear at chosen positions and distances relatively to the fly without significant delay (Strauss et al. 1997, J exp Biol 200:1281-96). In the first phase of an experiment the fly under test was allowed to approach a given LM. As soon as it crossed the center of the platform the position of the LM was instantly changed to lateral positions between 25° and 165° on the retina. The delay time from switching the LM position to a change in the fly's course was recorded.

(1) When approaching a straight-ahead LM which is suddenly switched to a lateral position, wild-type flies continue walking towards the former position of the LM for some time. If the position had switched less than 100° to the side the fly will finally change its course towards the new LM position. If the position had switched more than 100° the fly is more likely to turn away from that LM. Avoidance becomes more likely with increasing angles and can reach up to 70%. (2) The time delay until the fly changes its course (either towards or away from the LM) is shortest when the switching angle is small. It almost doubles when the LM switches to a very rear retinal position. (3) Moreover, when the switched LM comes closer, the delay time to course correction is markedly shorter.

Our model of the mechanisms underlying LM orientation behavior assumes that the fly integrates visual motion *en route* in four or more independent compartments of the visual field. If a certain threshold is reached in one of the compartments the fly will either turn towards or away from that particular LM. Because frontal eye parts receive less parallax motion than lateral eye parts a weighting function is introduced which puts higher weight on frontal than on lateral motion detectors. The model reproduces many known behavioral idiosyncrasies of the fruit fly. We are currently testing the model on a mobile robot. (Supported by BMBF, FKZ: 0311855)

The minimum angle for the color discrimination in the butterfly

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The Japanese yellow swallowtail butterfly, *Papilio xuthus*, has true color vision, which is based on some or all of the six classes of spectral receptors identified in their eyes. The spectral receptors are embedded in the ommatidia, building blocks of the compound eye. In fact, the *Papilio* ommatidia can be divided into three types, each containing different sets of spectral receptor classes. The different types of ommatidia distribute rather randomly in the eye: the eye of *Papilio* is a random mesh of spectrally heterogenous units. How do the butterflies see color with such eyes? How many ommatidia are necessary to recognize color? We tackled this question by measuring the minimum visual angle required for color detection.

For this purpose we established a behavioral protocol using a Y-maze. The Y-maze apparatus consists of an entrance area (50w x 50h x 50d cm), two arms (30w X 50h x 50d cm), and a decision area that connects the entrance area and the arms. The apparatus was assembled so as to the butterflies in the decision area can see both visual targets presented in two arms simultaneously. We first trained butterflies to feed on nectar in front of a disk of certain color presented vertically in the entrance area. We then presented two disks on the far end walls of two arms, and let the trained butterflies to select one of the two disks. We changed the diameter and color of the disks, and measured the butterflies' discrimination ability.

We trained butterflies with blue, green and red. In the test, we presented a gray disk and a disk of the trained color for the indivisual in each arm. Both color disk and gray disk have identical size and subjective brightness. The diameter of the disk was changed between 0.6 and 5 cm (corresponding 0.5 - 4 degree). When the butterflies once crossed the imaginary border between the decision area and one of the arms, we scored the behavior as one visit to the target presented in the arm. We evaluated that butterflies discriminated the color from gray, only when the ratio of the visits to the color exceeded 60%. As a result, the minimum visual angle appeared to be around 1 degree in all colors. This is much smaller compared to the detection limit in the honeybee, which is 5 degree. The1 degree visual angle in the *Papilio* eye roughly corresponds to the acceptance angle of an ommatidium (1.2 deg) and the interommatidial angle (0.8 deg). The coincidence of the detection limit and the optical resolution limit implicates that the multiple spectral receptors coexist in single ommatidia may construct independent color detection units.

499 Path integration in desert ants, *Cataglyphis*: Redirecting global vectors

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Desert ants, *Cataglyphis*, use path integration as their predominant mode of navigation. They do so by integrating all angles steered and all distances covered into one global vector. The state of this vector is updated during the entire foraging trip and is reset to zero after the ant has arrived again at the starting point, the nest. In addition, landmark routes can guide even "zero-vector" ants (i.e. ants which, within their working memory, have reset their global vector to zero state) from the food source to the nest. Here we show that such zero-vector ants, which have travelled 1-3 times from the food source along an artificial landmark route to the nest but not back to the food source, exhibit a "negative global vector" when released in a landmark-free test area. The question arises whether and how this newly acquired global vector interacts with the former global vector. Further investigation will try to clarify this by an analysis of the outbound runs of ants which had entered the nest with their manipulated global vector.

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500 Beacon versus vector navigation in homing ants, *Cataglyphis fortis*

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Homing desert ants, Cataglyphis fortis, use piloting to supplement their vector navigation (path integration) system for pinpointing a goal, e.g. their nest entrance. In previous experiments it has been shown that skyline landmarks specifying the goal by their geometrical arrangement come into play only when the ants are close to the goal (Michel and Wehner, 1995: Proc. Neurobiol. Conf. Göttingen 23: 41, Wehner, Michel and Antonsen, 1996: J. Exp. Biol. 199: 129-140). Now we investigate whether the same holds true for landmarks used as beacons. For this the nest from which the experimental ants were taken was provided with a cylindrical landmark positioned right at (closly behind) the goal.

In particular, a feeder was established 15m to the south of the nest, at which the landmark was positioned 10cm to the north of its entrance. Trained ants were caught at the feeder and displaced to a test field with a 1-m square recording grid of lines. There, in a parametric series of experiments, the landmark was positioned at various locations relative to the ants point of release: near to the fictive position of the nest, i.e. 3m to the left or right of it, as well as near to, i.e. 3m to the left or right of, various states (100%, 67%, 33%) of the global home vector, or even near to the point of release, i.e. 5m north of it.

The experiments showed that beacon landmarks were a dominant factor in the navigation system of Cataglyphis. In nearly all cases the ants exhibited distinct deviations from their vector-based path towards the landmark. Beacon orientation overruled vector navigation even if only one third of the homebound vector had been run off.

In conclusion, Cataglyphis uses landmarks in different ways depending on whether landmarks indicate the goal directly (as a beacon) or indirectly (as a panoramic skyline). In case of skyline landmarks the ants activate their memory for landmarks only when the path-integration vector has been run off nearly completely, while the memory for beacon landmarks can already be activated shortly or immediatley after the ants start for their way home.

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Involvement of NMDA receptors in normal retinal development

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NMDA receptors play important roles in neuronal survival and differentiation as well as in the formation and stabilization of synapses and circuits during nervous system development. To investigate the function of NMDA receptors in retinal development, we have studied a gene targeted mouse deficient in the mandatory NMDA receptor subunit NR1 (NR1 -/-).

NR1 -/- mice die at birth. To study postnatal retinal development, retinal explants, dissected at E19, were cultured in a roller incubator (organotypic culture). After 15 days *in vitro* (DIV 15), retinal gross anatomy, expression of synaptic markers, neurotransmitter receptors, and the morphology of the different retinal neurons was examined with immunocytochemistry in NR1 wild-type (WT) and NR1 -/- retinal cultures.

At E 19, no anatomical differences were noted between the NR1 -/- and the WT retinae. At DIV 15, the NR1 -/- mice, like the WT mice, had a developed retinal architecture with the characteristic lamination of three nuclear and two plexiform layers. In the NR1 -/- retina, however, the outer and the inner nuclear layer were significantly thinner compared to WT retinae. Lack of functional NMDA receptors had no obvious effect on the cellular development of amacrine, horizontal and ganglion cells and the formation of inhibitory GABAergic and glycinergic synapses. The glutamatergic system in the NR1 -/- retina was greatly perturbed, as seen in a lower number and changed distribution of glutamatergic synapses in the plexiform layers. Rod bipolar cell number was significantly reduced, and their morphology changed, in the NR1 -/- retina. Rod bipolar cells develop in the NR1 -/- culture, as shown with BrdU staning, but they undergo increased apoptosis between DIV 8 to DIV 15 (TUNEL).

These findings suggest that lack of functional NMDA receptors in the developing retina does not globaly effect neuronal and synaptic differentiation. It selectively perturbs the

development of the glutamatergic system, and that of the rod bipolar cells by increased apoptosis.

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502 Dendritic field size correlates with glutamate receptor expression in amacrine cells of mouse retina

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The mammalian retina contains over 20 morphological varieties of amacrine cell types, making up the most diverse population of all retinal cells. These interneurons receive excitatory glutamatergic input from bipolar cells and provide GABA- and glycinergic inhibition to other cells in the retina. Amacrine cells exhibit widely varying light evoked responses which are in large part defined by their pre-synaptic partners. We wondered whether differential expression of glutamate receptors (GluRs) among amacrine cells might underlie some of this functional diversity.

We performed both whole cell patch-clamp and single cell RT-PCR experiments on amacrine cells in mouse retinal slices. For the electrophysiological characterization of the GluRs, we used selective agonists and antagonists to discriminate responses mediated by NMDA/ non-NMDA (*NBQX*) and AMPA/ KA receptors (*GYKI 52446*, *cyclothiazide* and *SYM 2081*). Cells were classified according to their dendritic field size into either wide field or narrow field cells after filling with Neurobiotin. As expected, all cells (n = 17) had good responses to non-NMDA agonists. In addition, we were able to elicit very small NMDA responses from wide field amacrines (50%), whereas narrow field amacrines showed no responses to NMDA. While *specific* AMPA receptor responses were obtained from all amacrines recorded, *specific* KA receptor responses could only be obtained from wide field cells (50%).

It is well known that narrow field amacrines preferentially use glycine as their neurotransmitter whereas wide field cells use mostly GABA. Using single cell multiplex RT-PCR, we studied the expression of GluR subunits among GABAergic (n = 9) and glycinergic (n = 8) amacrine cells. Both populations expressed AMPA receptors, but while GABAergic cells preferentially expressed GluR 1 & 4, glycinergic cells preferentially expressed GluR 3 & 4. In contrast to the pharmacological data, KA receptor subunits were more commonly amplified from glycinergic than GABAergic cells. Finally, while NR1 subunits were commonly expressed in both populations, NR2 subunits were more often expressed by GABAergic cells.

The data we have collected, using two independent but complementary techniques, suggest that AMPA, KA and NMDA receptors are differentially expressed between narrow and wide field amacrine cells. The fast kinetics of AMPA receptors are thought to be able to follow very high temporal frequencies, whereas slower desensitizing KA and NMDA receptors might underlie sustained signaling. Selective expression of kinetically different glutamate receptors among amacrine cell types may be involved in generating transient and sustained inhibitory pathways in the retina. It is interesting to note

that since AMPA and KA receptors are not generally clustered at the same post-synaptic sites, a single amacrine cell which expresses both AMPA and KA receptors may provide inhibition with different temporal characteristics to individual synaptic partners.

Dominance of short-wave sensitive cones in the retinae of subterranean African mole-rats (Rodentia, Bathyergidae) 503

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Mammalian retinae contain rod and cone photoreceptors. Most mammals are cone dichromats, having two types of cone opsins (Jacobs, Biol. Rev. 68: 413-471, 1993). The majority of cones are L-cones with a peak sensitivity at longer wavelengths (green to red, depending on species); a minority of about 10% are S-cones with a peak sensitivity at shorter wavelengths (blue or near UV, depending on species). Only relatively few species deviate from this general pattern. We have studied the photoreceptor equipment, and in particular the presence of spectral cone types, in subterranean mole-rats of the bathyergid family, for which light and vision is of little importance. The subterranean blind mole-rat *Spalax ehrenbergi* of the murid family has an L-cone opsin, but no S-cone opsin (David-Gray et al., Nature Neurosci. 1: 655-656, 1998 and EJN 16: 1186-1194, 2002). We were interested to see whether other subterranean rodents show the same pattern.

The present study includes three species of Bathyergidae (African mole-rats), Ansell's mole-rat *Cryptomys anselli*, giant mole-rat *Cryptomys mechowi*, and naked mole-rat *Heterocephalus glaber*. All animals came from our breeding colonies. Eyes were obtained from individuals sacrificed for an unrelated project. Retinae were fixed in paraformaldehyde or Bodian's fixative. Spectral cone types were assessed using the antibodies JH492 and COS-1 against L-cone opsin, and JH455 and OS-2 against S-cone opsin (kindly provided by J. Nathans and A. Szél, respectively). Rods were assessed by conventional histology. All three species have rod-dominated retinae but possess significant cone populations. A quantitative assessment in *C. anselli* revealed some 120,000-140,000 photoreceptors/mm^2, of which some 12,000-14,000/mm^2 (about 10%) were cones. In all three species, nearly all cones are strongly reactive to the S-opsin markers. The L-opsin markers produce very faint labeling barely above background. Only a few cones per retina show exclusive L-opsin label, but most of the strongly labeled S-cones also faintly label for L-opsin (potential dual pigment cones).

In summary, S-opsin dominates and L-opsin appears to be present at very low levels in the cones of bathyergid mole-rats. In contrast, the murid blind mole-rat *Spalax* possesses the L-opsin and lacks the S-opsin. Evidently, within the order Rodentia an adaptation to fossorial life is compatible with very different spectral cone properties. These findings suggest that colour vision is impeded in both groups, but that their 'spectral windows' differ. The functional role of the cones in subterranean species remains to be determined.

504 Selective synaptic expression of complexin I/II in the mouse retina

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Complexin I (CPX I) and Complexin II (CPX II) are small, soluble proteins that bind to the assembled exocytotic core complex, and are involved in the regulated release of neurotransmitter at synapses. Homozygous CPX I/II double knockout mice die within a few hours after birth because of a severely compromised synaptic function (Reim et al., Cell 104: 71-81, 2001).

With immunocytochemistry and confocal laser scanning microscopy, we examined in single and double labeling experiments the cellular, synaptic and developmental expression of CPX I/II in wild-type mouse retina. Furthermore, we analyzed in retinae of single mutant mice, the effects of lack of CPX I or CPX II on neuronal and synaptic development.

CPX I/II staining is present in the inner plexiform layer with several strata of higher and lower immunoreactivity. In addition, horizontal cell processes in the outer plexiform layer and somata of amacrine and ganglion cells in the inner nuclear layer and the ganglion cell layer are labeled for CPX I/II. Double labeling experiments demonstrate the expression of CPX I/II at GABAergic synapses of amacrine and horizontal cells, and their absence at glutamatergic and glycinergic synapses. During postnatal retinal development, CPX I/II immunoreactivity is first seen between postnatal day 2 (P2) and P4 in the inner plexiform layer, when amacrine cell synapses are being formed (around P3). Analysis of retinae from CPX I and CPX II single knockout mice, reveales that retinal architecture, development of retinal neurons and synapses, and amacrine cell synaptic ultrastructure is not affected by the mutations.

The selective expression of CPX I/II at GABAergic synapses in the retina implicates that the glycinergic amacrine cell synapses and the glutamatergic ribbon synapses of photoreceptor and bipolar cells will express CPX isoforms other than CPX I/II. This is further evidence that the molecular composition of the different synapses in the retina, GABAergic, glycinergic, glutamatergic, etc., is different. Surprisingly, lack of CPX I/II seems to have no effect on the cellular and synaptic development of the retina.

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Color Contribution to Spatial Information Processing and to Contrast Detection During Background Illumination in the Turtle Retina

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SCIENTIFIC BACKGROUND: Physiological and morphological evidence suggest that the turtle retina contains one of the most complex cone systems among vertebrate and therefore, the turtle visual system has the potential for sensitive color vision. However, the properties of color vision depend also upon the neuronal interactions in more central stages of the visual system. The first visual neuron to exhibit color opponent behavior is the chromaticity horizontal cell (CHCs), which receives antagonistic inputs from cones of different spectral types. These horizontal cells are considered to be crucial for center-surround antagonism in bipolar and ganglion cells and thus, play a dual role in spatial and color information processing. Our goals were to study spatial-chromatic interactions in the C-type horizontal cells and to examine the potential contribution of color to contrast detection during background illumination.

METHODS: Photoresponses were recorded with intracellular microelectrodes from cone photoreceptors and C-type horizontal cells in the everted eyecup preparation of the turtle Mauremys caspica. The intensity, color and spatial properties of the light stimuli were changed while illumination the retina with chromatic background light of different wavelength and intensity.

RESULTS: Spatial-chromatic interaction: When bright light stimuli of “neutral” wavelength (spectral location where response polarity reverses) were used, the photoresponses of CHC were of hyperpolarizing pattern with full field illumination but changed into a depolarizing one when a small spot or a thin annulus were used. This observation was found to reflect differences in the receptive field properties of CHC for the depolarizing and hyperpolarizing components of their photoresponses.

Chromatic adaptation: The voltage range of operation of CHC was either reduced or augmented depending upon the wavelength of the background and that of the light stimuli, while the sensitivity to light was decreased by any background. The contrast-response relationship, derived from the peak amplitudes of the photoresponses, was nearly unchanged when the wavelength of the light stimulus and that of the background were similar. However, when the wavelength used for background and stimulus were opponent, contrast-response curves became steeper as ambient illumination was made brighter. Comparing the effects of iso-luminant backgrounds on each of the opponent inputs to the Red/Green C-type horizontal cells indicated that the location of the intensity-response curve of these cells along the intensity axis (value of semi-saturation constant) could be accounted for by light adaptation mechanisms in the cone outer segments. However, background desensitization also depended upon three processes proximal to the outer segment: (1) voltage range of operation (response compression/expansion), (2) pre-synaptic [cone]- induced transmitter release and (3) post-synaptic [horizontal cells] –desensitization.

CONCLUSIONS: Color is an important factor in visual information processing within the distal retina of the turtle. It can alter the spatial pattern detection and contribute to sensitivity during background illumination.

506 Blockade of retinal nicotinic but not muscarinic receptors impairs whole field motion perception in goldfish

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Motion vision is one of the most important visual abilities. As motion coding begins in the retina the relevance of retinal neurotransmitters on motion perception should be investigated. The Optomotor Response (OMR)- a reflex-like behavior of many animals evoked by moving whole-field visual stimuli – can be used to measure motion vision. Retinal Acetylcholine has been shown to play a major role in retinal motion coding. Thus nicotinic and muscarinic receptor antagonists were applied to the retina of goldfish and changes in their OMR recorded.

The OMR was elicited by a rotating (18, 30, 60 and 78 deg/s pattern velocity) pattern cylinder consisting of white stripes and inter-stripe spaces (each 2 cm) in front of a black background. Goldfish followed the rotating pattern and adjusted their swimming velocity to the pattern velocity. Rounds swum in pattern direction were counted as a positive OMR and rounds swum against the pattern direction were subtracted from them. Results were recorded as OMR in rounds per minute. The OMR was tested before and after the injection of Curare (intraocular concentrations: 3, 12, or 36 mikroM) and/or Atropine (100 mikroM). After the injection of Curare, angular velocities were determined to control for motor effects. Neither the vehicle nor Atropine had any effect on the OMR. Curare reduced the OMR in a concentration-dependent manner: 3 mikroM had little to no effect, 12 mikroM reduced the OMR by up to 50% at 60 and 78 deg/s and 36 mikroM reduced the OMR by 50% at 18 and 30 deg/s and eliminated it at 60 and 78 deg/s pattern velocity. Data were excluded once an animal developed a substantial loss in maximal angular velocity during an experiment. Three animals received

injections of Curare (10 mikroM) and Atropine (100 mikroM). The results were similar to the 10 mikroM Curare injections, i.e. Atropine did not change the Curare induced reduction of the OMR.

Acetylcholine seems to be essential for retinal processing of moving whole field stimuli and thus for motion vision. An effect of Curare on motor activity does not seem to be responsible for the observed loss of the optomotor response. As Atropine was unable to reverse the effect of Curare it is proposed that nicotinic receptors are important for retinal motion processing but muscarinic receptors are not involved.

2-Photon-imaging of chloride transients in ON-type bipolar cells in a transgenic mouse retina expressing 'Clomeleon' 507

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Bipolar cells are retinal interneurons that provide the inner retina with photoreceptor signals. One major functional subdivision is into ON and OFF bipolar cells which, when stimulating their receptive field center with light, respond to light onset or offset, respectively: Bright stimuli depolarize ON bipolar cells and hyperpolarize OFF bipolar cells.

In addition, bipolar cells have an antagonistic center-surround receptive field; surround illumination generates hyperpolarization in ON bipolar cells and depolarization in OFF bipolar cells. The dendrites of bipolar cells are located in the outer plexiform layer (OPL), where they receive glutamatergic input from photoreceptors and presumably GABAergic input from horizontal cells which is thought to help forming this antagonistic center-surround receptive field. Horizontal cells are OFF cells such that maximal release of GABA and therefore maximal inhibition in the bipolar cells occurs in darkness. Thus, the presence of GABA receptors in both ON and OFF bipolar cells is puzzling, because GABA receptor mediated inhibition would only "make sense" in the case of OFF bipolar cells. In these cells inhibition would help to create an antagonistic receptive field organization, while in ON bipolar cells it would diminish the excitatory center response (at least for an "optimal" antagonistic stimulus like a small bright spot on a dark background).

Recently, an immunohistochemical study of the chloride transporter distribution in bipolar cells revealed that ON bipolar cell dendrites specifically express a chloride accumulating type of transporter, suggesting that the intracellular chloride concentration is higher in their dendrites than in their synaptic terminals [1]. A chloride reversal potential that is more positive than the resting potential would allow the GABA receptors to mediate excitation, and hence provide ON bipolar cells with the "correct" surround input for an antagonistic receptive field. Patch-clamp studies, however, suggest that the intracellular chloride concentration is not significantly higher in the dendrites than in the axon terminals where GABA is known to mediate inhibition, and that GABAergic input at the dendrites might generate hyperpolarization rather than depolarization [2, 3].

To address this problem, we have optically recorded chloride signals from a subpopulation of ON bipolar cells that express the ratiometric biosensor Clomeleon [4] in transgenic mice [5]. Unlike patch-clamp recordings optical imaging allows chloride signals in fine dendritic processes to be monitored without perturbing the intracellular ionic composition. Preliminary results indicate that the bipolar cells expressing Clomeleon are functionally intact and that application of GABA generates significant changes in fluorescence intensity. Ratiometric measurements will make it possible to map the local chloride reversal potential and thereby predict the polarity of GABAergic input. The observation of local dendritic chloride signals will lead to a better understanding of an important mechanism that mediates lateral inhibition in the OPL.

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508 Separation of spatio-temporal receptive fields into sums of amplitude modulated Gaussian components

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Visual cortical simple cells have been experimentally shown to reveal non-trivial dynamic receptive fields (RFs) comprising different phases of specifically tuned enhanced and suppressed activity [1]. A recently developed analytical method based on nonlinear neural field models for describing such dynamic responses suggests that the space-time profile of a simple cell should be approximately separable into a sum of temporally amplitude modulated Gaussian spatial components [2]. The theory relates each single component to a particular anatomical projection between the underlying cell classes that shape the response (e.g., LGN cells, excitatory and inhibitory cortical neurons). In the present work, we investigate this possibility by means of numerical fits of sums of Gaussians to response functions observed in experiments and computer simulations. This way the relative contribution of feedforward and cortex-intrinsic excitatory and inhibitory feedback mechanisms to single cell tuning can be approached and quantified in experimental data.

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509 The morpho-functional organization of the retina of the elephantfish (*Gnathonemus petersi*)

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The retinae of five adult elephantfish (Gnathonemus petersi) were studied by light and electron microscopy, including confocal microscopy. The retina of this species is characterized by a very thick layer of photoreceptor segments which are arranged in bundles, each surrounded by a cup-like, complex 'sheath' arising from the retinal pigment epithelium (RPE).

There are two observations suggesting that the photoreceptors within such a bundle constitute a functional unit rather than individual image detectors; (i) the thick cup-like RPE-derived sheaths display light-reflecting guanin crystals which will keep any light within a given bundle but will distribute this light to many if not all photoreceptors of this bundle; and (ii) the short and thin outer segments of cones are compressed within

the inner core of the bundle where they occupy not more than about 15% of the available retinal cross-sectional area.

Assuming that the photoreceptor bundles (about 600 per mm² retinal surface area) are functional columnar units, the constituents of an average unit were quantitatively assessed throughout the outer and inner retina. Such a unit contains about 25 cones and 600 rods, which transmit visual information to about 30 neurons of the inner nuclear layer (INL) and, finally, to about 4 ganglion cells. The absolute densities of cones (about 20,000 /mm²) and rods (about 350,000 /mm²) are in the upper range of the values found among various vertebrates. The availability of one ganglion cell for some 6 cones, however, is in the lower vertebrate range. The presence of hardly more than one neuron in the INL (i.e., less than one bipolar cell!) per cone even represents an exceptionally low value, particularly for freshwater fish retinae where normally six or more INL neurons per cone are found. These data argue in favour of an extreme convergence of visual signals, and of the above-mentioned hypothesis that all cones of a bundle, rather than every single cone, act as the smallest functional unit for visual signal processing. This seems to be comparable to the situation of the insect compound eye where the ommatidia (each comprised of 8 photoreceptors), rather than the individual photoreceptors, constitute the smallest functional units.

This type of retinal organization is clearly not optimized in respect to spatial resultion. However, it is also unlikely that the elephantfish retina is optimized in respect to photon capture / light sensitivity, as the outer segments of both cones and rods are short and thin, and thus do not provide a large membrane area available for the insertion of opsin molecules. Rather, this retina may be specialized to detect (moving? / large?) objects within an extremely rough-screen image as provided by the turbid water where the fish live in. It is here speculated that only large "relevant" objects (e. g., predators) are detected whereas the rich background of small "irrelevant" environmental constituents (such as decaying plant or animal debris, and gas bubbles) is 'filtered out' of the image.

Improved underwater vision in humans **510**

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The human eye is generally considered an example of a terrestrial eye. We need the curved outer cornea to be in contact with air if the image is to be focused properly on the retina. If a human eye is submersed in water we lose more than two-thirds of our refractive power and thus the image becomes severely blurred.

However, some populations of sea-gypsies in Southeast Asia seem to manage quite well under water. The children of these tribes dive with an unaided eye and search for small food items on the sea floor. We suspected that these people might have adapted in some way to improve their underwater vision.

We examined six children from a tribe of Moken sea-gypsies in Thailand, using European children on holiday with their families as a control group. The results clearly show that the Moken children see better under water, being able to resolve gratings more than twice as fine under water compared to European children (Moken children (n=6):

6.06±0.59 cycles/degree, European children (n=28): 2.95±0.13 cycles/degree; Mann-Whitney test: $p<0.001$).

When we investigated the pupils of the children we discovered the explanation for this improved underwater acuity. We found that the Moken children closed their pupil under water much more than the European children did (Moken children: 1.96±0.05 mm, n=6; European children: 2.50±0.05 mm, n=15; Mann-Whitney: $p<0.001$). On land both groups have the same pupil size (Moken children: 2.33±0.06 mm, n=6; European children: 2.30±0.04 mm, n=15; Mann-Whitney: $p=0.48$).

The smaller pupil size of the Moken children combined with accommodation explains the improved underwater acuity. In fact, the Moken children accommodate as much as they possibly can (almost 16 D, which is the limit for children of their age). The reaction of the European children is quite the opposite – their pupil size stays more or less constant and they do not accommodate. Since a severely blurred image usually triggers very little accommodation or none at all, this is a quite normal response. We also found that the pupil constriction and accommodative response observed in Moken children seems to be a reflex elicited by water, since these children also have a similar response when diving with goggles.

Improved underwater vision in Moken children has clear adaptive value for the lives which they lead, but how have they achieved it? One possibility is that the Moken have learned to accommodate voluntarily due to the extensive use of their eyes in water. It should then be possible for all humans to learn to see better under water. Preliminary results show that this is indeed the case. European children can learn to see better under water, though accommodation comes in burst and is not sustained through the whole diving period (as with the Moken children).

511 The influence of dopamine on temporal transfer properties in the goldfish retina examined with the ERG

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Introduction: Temporal resolution in goldfish is modulated by dopamine through a D2-R mechanism as demonstrated in behavioural experiments Mora-Ferrer & Gangluff, in press). However, little is known, and understood, about the localization and function of D2-R in fish retina. The effect of specific D1- and D2-R antagonists on temporal transfer properties (TTP) of the goldfish retina was investigated using the ERG. Methods: Photopic ERG were measured in the vitreous and at various retinal depths before and after intravitreal injection of sulpiride, SCH 23390 or aspartate. Aspartate was used to determine potential effects of either of the dopamine antagonists on the photo receptor potentials. Drug concentrations of sulpiride and SCH 23390 and the experimental procedure were similar to the behavioural experiments. Stimuli were a flickering light (2-50 Hz) for the overall TTP and single light-pulses (100 ms) for intra-retinal ERG measurements. Results: The amplitude-frequency plot of the ERG prior injection shows a low pass characteristic with an upper corner frequency ? around 25 Hz. After injection of sulpiride ? shifted by about 25-30% to lower frequencies whereas SCH 23390 had no

effect. Nevertheless, both sulpiride and SCH 23390 changed the single pulse response properties from all retinal depths recorded. Isolated photo receptor response was not altered by sulpiride. Discussion: Sulpiride changed, through a D2-R mechanism, retinal TTP as determined from the ERG. The isolated a-wave was not affected by sulpiride. Single-pulse measurements at different retinal depths revealed that both D1-and D2-R antagonists had substantial effects in altering the response properties. SCH 23390 had its? largest effects in the ganglion cell layer, but surprisingly did not change the overall TTP. This corroborates the results from the behavioral experiments on temporal resolution. The effect of sulpiride was largest, for single-pulses, in the inner retina especially on the OFF-part of the ERG. The ERG data for the overall TTP and from single-pulse experiments indicate a D2-R modulated mechanisms involved in temporal coding properties mainly in the inner retina. Although large changes to cell responses to single-pulses could be observed after blockade of D1-R overall TTP remained unchanged. Therefore it seems possible that D1-R are not involved in temporal processing but other processing properties.

Molecular analysis of the supramolecular Usher 1 protein complex in the neuronal retina 512

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Human Usher syndrome (USH) is the most common form of deaf-blindness. 3 clinically distinct Usher types (USH1, 2, 3) exist. Usher type I (USH1), the most severe form, is characterized by profound congenital deafness, constant vestibular dysfunction (balance deficiency) and prepubertal-onset retinitis pigmentosa (retinal degeneration). Seven USH1 genetically heterogeneous subtypes USH1A-G have been obtained, and four of the corresponding genes have been identified: USH1B encodes the actin-based motor protein myosin VIIa, suggested to participate in intracellular transport and endo- and exocytotic processes. USH1C encodes harmonin, a protein that contains PDZ domains, known as organizers of supramolecular protein complexes. Mutations in the genes encoding two cell-cell adhesion proteins, cadherin 23 and protocadherin 15, underlie USH1D and USH1F, respectively. However, the functions of the proteins underlying USH1 remained almost elusive.

The aim of our project is to analyze the molecular and cellular function of the USH1 proteins in the vertebrate retina. Over the last years we have gathered essential information on the function of myosin VIIa (USH1B) in the retina by the analysis of mouse models and the identification of mysoin VIIa interacting proteins. In the cells of the retinal pigment epithelium myosinVIIa contributes to the delivery of melanosomes and in photoreceptor cell it participates to the transport of the visual pigment opsin. More recently, after identification USH1C, we have concentrated on the characterization of the PDZ-domain protein harmonin, a putative protein complex organizer. *In vitro* F-actin binding assays and transfection studies reveal that harmonin is a potent actin bundling protein. Further protein-protein interaction assays (GST-pull downs, and co-immunoprecipitations) and co-transfections reveals that harmonin indeed acts as a scaf-

fold protein for USH1 proteins binding myosin VIIa (USH1B) via its PDZ-1 and cadherin 23 (USH1D) via its PDZ-2. All three proteins are expressed in the neuronal retina. Immunocytochemistry and the biochemical analysis of photoreceptor fractionated compartments indicate that all members of this USH1-complex co-localize at the ribbon synapse of photoreceptor cells in the vertebrate retina. At this specialized synapse the USH1-complex may contribute to actin-based cortical cytoskeletal matrices of the pre- and postsynaptic regions which are thought to play a fundamental role in the organization, structure and function of the synaptic junction. The scaffold protein harmonin may also functionally bridge the motor activity of myosin VIIa involved in endo- or exocytotic processes at the synapse with the cell-cell adhesion complex generated by cadherins (e.g. cadherin 23) and other transmambrane proteins (e.g. the myosin VIIa interacting protein vezatin). Adhesion molecules are supposed to hold the synaptic cleft in close register. Dysfunction of any of the USH1-complex partners may result in synaptic dysfunction causing retinitis pigmentosa, the clinical USH type 1 phenotype in the retina of human patients.

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513 Directionally selective units in the goldfish retina: A colour-blind mechanism driven by two spectral classes of cones

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Responses of directionally selective ganglion cells (DSGCs) of 4 classes (ON- or OFF-types, preferring rostro-caudal or caudo-rostral movements, in terms of outer space relative to the fish) were recorded extracellularly from their axon terminals in the superficial layer of the tectum opticum of immobilised fishes. When studied by the method of "paper colourimetry" (moving coloured papers of known reflectances over coloured background under controlled ambient illumination), they proved to be colour-blind and sensitive to far red light. In experiments with the computer-controlled colour monitor this colour-blindness was confirmed at least for low colour contrasts. Coloured stimuli moved in preferred direction over some fixed background can be divided into four groups according to response they elicit, which in turn depends on position of stimulus colour in the goldfish colour space: stimuli that are darker then the background, i.e., that evoke responses both to their introduction into the receptive field (RF) of the OFF-type DSGCs or to withdrawal from the RF of the ON-type DSGCs; (2) stimuli that are brighter then the background, i.e., that evoke opposite cell responses; (3) stimuli that match to the background, i.e., do not evoke response neither to their introduction in, nor to withdrawal from the RF, and (4) stimuli (usually of high colour contrast unachievable by means of painted papers) that evoke small response to movement in any direction, in other words the DSGCs lose their directional selectivity. These two last groups cover a narrow layer it the goldfish colour space between regions of dark and bright stimuli. Orientation of this layer permits to estimate contribution of different cone mechanisms to spectral sensitivity of DSGCs, which was found to be determined not only by red

cones but by green ones also participating weakly in opponent manner. As a result, the spectral sensitivity of DSGCs is reduced in the blue-green end of the spectrum, its maximum being shifted even further to the red end. This spectral sensitivity shift may be considered as adaptation to aquatic environment, in which acute vision is in general possible only in long-wavelengths due to substantial light scatter in blue-green region of spectrum.

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Identification and characterization of retinoic acid-binding proteins in the carp retina 514

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Retinoic acid is a well known morphogenetic factor responsible for a variety of organisational aspects during development. It acts through activation of nuclear receptors which in turn activate retinoic acid response elements regulating transcription.

In the vertebrate retina, all trans-retinoic acid (at-RA), besides being a morphogenetic factor determining the dorsal/ventral axis during development, it also acts as a light-dependent neuromodulator. at-RA induces adaptational effects in retinal horizontal cells that underlie light adaptation. It promotes the protrusion of spine-like structures from the terminal dendrites of horizontal cells involved in the feedback pathway between cones and horizontal cells and regulates the conductivity of gap junctions between horizontal cells. These modulatory effects take minutes and persist in the presence of actinomycin ruling out transcriptional effects, and the molecular mechanisms of its action are still unknown. Recent findings in fish retina (Zhang & McMahon, 2000, PNAS 97: 14754) suggest that at-RA binds to an external membrane binding site, which activates an intracellular signal transduction pathway. We therefore studied the distribution of retinoic acid receptor α-like (RARα-like) proteins in the carp retina, and the existence of putative at-RA-binding proteins in carp retinal membranes.

In order to reveal specific at-RA-binding, a receptor binding assay with different retinal fractions of either cytosolic or membrane proteins was carried out using tritiated at-RA ([3H]at-RA) as ligand. Furthermore, a photo-affinity-labeling assay and SDS gel electrophoresis were used to label and separate [3H]at-RA-binding proteins in these fractions. The distribution of RARα-like proteins in the carp retina was analyzed by means of immunochemistry using RARα antibodies.

Specific binding of [3H]at-RA was measured in membrane fractions (KD = 37,7nM) as well as in cytosolic fractions (KD = 21,3nM) of the carp retina. Photo-affinity-labeling and autoradiography revealed the existence of several at-RA-binding proteins in the cytosolic fraction and 6 different at-RA-binding proteins in the membrane fraction. One of these proteins had a molecular weight of 43 kDa and subsequent immunochemistry revealed a RARα-ir protein with the same molecular weight in the membrane fraction and in isolated horizontal cells. In retinal cryosections of the carp retina, application of the same antibodies resulted in a prominent, punctate labeling of the outer plexiform layer and in addition, isolated horizontal cells were also strongly labeled.

In summary, the carp retina reveals different at-RA-binding membrane proteins. One of these proteins co-migrates with a RAR α-like protein enriched in membranes and horizontal cells, suggesting that horizontal cells express a membrane-bound at-RA-binding protein.

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515 Stimulus velocity reconstructed from retinal ganglion cell activity using Bayes' method

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Statistical estimation theory has recently been attracting attention in the Neurosciences as it has proven useful to investigate neural coding. In particular, stimulus reconstruction by Bayesian inference has been successfully applied, for example to reconstruct the position of a moving rat from recordings of the animal's hippocampal place cell activity [1]. Here, Bayesian inference is used to estimate stimulus movement parameters such as velocity and acceleration from the activity of a retinal ganglion cell population. The quality of the reconstruction will indicate which stimulus properties are transmitted by the retina, even by a relatively crude signal as the overall activity of a group of ganglion cells.

Ganglion cell action potentials in an isolated turtle retina were recorded with a multielectrode array [2]. The retina was optically stimulated with a moving square wave grating, its velocity changing rapidly every 500ms. After such a change, movement remained continuous at the new velocity until the next speed step. A sequence of 600 velocity changes was randomly generated and presented repeatedly. By pooling action potentials from 50 recorded neurons, instantaneous population firing rates were determined and subsequently combined with the corresponding movement velocities to calculate the relative probability of each response given a certain stimulus. Furthermore, the *a priori* distribution of stimuli was computed from the random sequence of actually chosen velocities. Applying Bayes' formula then results in a probability function, which, once known, enables the calculation of the stimulus that most probably caused any of the measured neuronal responses. Doing this for a sequence of firing rates allows a comparison between the actual and the estimated time course of velocities.

Infering the grating's velocity from the instantaneous ensemble ganglion cell firing rate works best for intermediate velocities, while high speeds are mostly underestimated. As the neuronal activity shows some fluctuations while stimulus speed is constant during the 500ms intervals, estimation is consequently spread around the actual velocity. Nevertheless, intervals with high velocity can clearly be distinguished from those with low stimulus speed. Additionally, the occurrences of strong increases in velocity can be well reconstructed. On average, the relative deviation between actual and estimated *speed increases* is minimal approximately 100ms following a change in velocity. In contrast, the difference between reconstructed and actual *velocity* is smallest approximately

200ms after the speed is altered. Thus, the most precise estimation of a speed increase is possible sooner than the most precise estimation of the speed itself.

In conclusion, even the rather crudely chosen mean response of all recorded ganglion cells regardless of their type still contains considerable information about stimulus properties and even enables statements about the timing of the retina's information transfer. Future work will also consider the neurons individually to find out whether there are certain ganglion cells that allow significantly better reconstruction of velocity or speed changes than others. Bayesian reconstruction is a tool that is predestined to accomplish this task.

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Light-dependent properties of retinal horizontal cells in wild type and rhodopsin knockout mice 516

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During the last years, the boundless possibilities of the transgenic techniques have made the mouse a favored subject in retinal research. Yet, little is known about the physiological properties of cells and pathways in the murine retina. The present study was therefore carried out to meet these needs with respect to the horizontal cells.

We adapted the everted eyecup preparation successfully used in studies on the non-mammalian retina to the mouse. In this preparation, the neural retina retains contact with the pigment epithelium, preventing photoreceptor bleaching and allowing for a prolonged retinal viability. During constant superfusion, intracellular recordings from horizontal cells were performed in combination with injection of the tracer Neurobiotin (2 % in 3 M KCl) which was afterwards visualized by Cy3-conjugated streptavidin.

The preparation was viable for about 5 hrs, and recordings from horizontal cells were stable for maximally 40 min. The cells displayed a dark potential of about ?30 to ?40 mV and performed the typical hyperpolarizing light responses with amplitudes of maximally 20 mV. Spectral sensitivity showed emphasis on short and middle wavelengths. The responsiveness declined following light adaptation and recovered during several minutes of dark adaptation. Tracer injection into a single horizontal cell soma resulted in an intense spread among the neighboring somata due to gap junctional communication. Tracer coupling was largest in a short-term (10 min) dark adapted condition (549 somata ± 168 S.D.; n=6) and was greatly attenuated following 1 hr of darkness (217 ± 69; n=5) or 15?20 min light adaptation (199 ± 61; n=5). This triphasic profile was also observed for the tracer spread among the axonal terminal arborizations of horizontal cells. In general, coupling between the axon terminals was considerably smaller than for the somata. Following short-term dark adaptation, tracer spread among the terminals was found over about 120 µm from the injection site which is only half the distance of the spread within the somatic network after prolonged darkness or light adaptation. Axonal terminal coupling was absent following prolonged dark adaptation or light adaptation. Under these conditions, the tracer diffused along the up to 500 µm long axon to the respective soma and was even found in some of the neighboring somata.

Horizontal cell coupling was also examined in rhodopsin knockout mice in which light sensitivity is mediated exclusively by cones. The general dynamics was similar to the wild type mice with intense somatic coupling following short-term dark adaptation (650 ± 22; n=3) and reduced tracer spread after prolonged darkness (360 ± 65; n=3) or light adaptation (184 ± 48; n=3).

These data demonstrate that gap junctional communication and light responsiveness of mouse horizontal cells are regulated by the ambient light conditions. The impact of the ambient light conditions still persists when the rod system is lacking indicating that the cone system is responsible for the regulating mechanisms.

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517 Localization of connexin36 to the outer plexiform layer of the mouse retina

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This study is aimed to identify the cellular source of connexin36 (Cx36) expression in the outer nuclear (ONL) and outer plexiform layer (OPL) of the mouse retina.

We generated transgenic mice that express (i) a fusion protein with enhanced green fluorescent protein (EGFP) targeted to the C-terminal domain of Cx36, and (ii) lacZ under the control of the Cx36 promoter. To determine the cellular location of Cx36, retinal neurons were identified immunocytochemically with established retinal markers and by intracellular injection of Alexa594. Cx36 could then be localized by targeting expression of EGFP and lacZ to the appropriate cell types in vertical sections and dissociated cells. In addition, we performed single cell RT-PCR with cytoplasm harvested from rod photoreceptors, horizontal cells, Müller cells, and pigment epithelial cells.

EGFP labeling in the OPL appears in discrete clusters, which are aligned but not coincident with cone pedicles. Cx36 is expressed by a population of OFF bipolar cells that are immunoreactive for neurokinin3 (NK3), and by a population of ON cone bipolar cells identified by the presence of the a-subunit of the G-protein Go. We found a close spatial relationship between Cx36 and the ionotropic glutamate receptor subunit GluR1, but not with GluR2/3, GluR4 and the kainate receptor subunits GluR5/6/7. Cx36 was never colocalized with CaBP 28K and protein kinase C, excluding expression of Cx36 in horizontal cells and rod bipolar cells, respectively. EGFP labeling in the ONL appears restricted to cones, whereas Müller glial cells and pigment epithelial cells do not express Cx36. The finding that only cones and cone bipolar cells express Cx36 was supported by the lacZ staining of isolated neurons and subsequently confirmed by the PCR data of identified cells. However, it is possible that the lacZ product is trapped to the endoplasmatic reticulum in the inner segment of rod photoreceptors, and thus Cx36 is also expressed in rods.

We have shown that Cx36 is expressed in dendrites of OFF bipolar cells which are aligned with cone pedicles. Interestingly, Cx36 and GluR1 appear to be closely associated in flat contacts made by OFF cone bipolar cells. Cx36 is also expressed in a popula-

tion of ON cone bipolar cells. In the ONL, Cx36 is expressed by cones, although we cannot rule out expression in rods at this time.

Cellular expression of connexin45 in the mouse retina 518

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In recent years it has become obvious that neurons in the adult nervous system make frequent use of gap junctions for their communication. Gap junctions are formed by channels, connecting directly two neurons, and each channel is a hexamer of connexins. Connexins form a family of proteins with presently 16 known members. The electrical synapses generated by gap junctions are modulated by voltage, pH, neurotransmitters and second messengers depending on their connexin composition.

In the retina, a wealth of gap junctions have been demonstrated histologically and some of these have been characterized also physiologically, but little is known about the involved connexins which is mainly due to the lack of specific connexin antagonists. The generation of transgenic mice offers a new approach to reveal the connexin composition of different gap junctions, and we have generated transgenic mice that express the lacZ reporter gene under the control of the Cx45 promotor. Retinal neurons were identified with established retinal markers in vertical sections and dissociated cells, and by intracellular injection of Neurobiotin in wholemount preparations. Cx45 was then localized by targeting lacZ expression to the appropriate cell types. In addition, single cell RT-PCR was performed with cytoplasm harvested from horizontal, bipolar and ganglion cells.

Cx45 is expressed in a subpopulation of ON cone bipolar cells identified by the length of their axons, but not in rod bipolar cells identified by the presence of PKC immunoreactivity. These results were confirmed by single cell RT-PCR of bipolar cells using corresponding primers. Horizontal cells do not express Cx45. Neurobiotin-labeled ganglion cells were classified according to the shape and size of their soma and the ramification pattern of their dendrites. Cx45 was expressed in bistratified ganglion cells with dendritic field diameters between 150 - 250 mu m. The two levels of stratification corresponded with the ON- and OFF sublaminae, respectively. Spiny and recursive dendrites of all orders were of uniform thin diameter. In many instances, tracer coupling to other ganglion cells was observed. Based on more detailed morphological characteristics, these bistratified ganglion cells can be subdivided into two subpopulations, one having all the features attributed to direction-selective ganglion cells. Cx45 expression was never co-localized with monostratified α ganglion cells exhibiting strong tracer coupling, an observation which was supported by single cell RT-PCR data.

We have shown that Cx45 is expressed in a subpopulation of cone bipolar cells and in bistratified ganglion cells which show homologous tracer coupling and share many similarities with direction selective ganglion cells.

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519 Caldendrin, a novel Ca^{2+} -binding protein, involved in synaptic plasticity in the fish retina?

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Light and dark adaptation of the teleost retina are accompanied by considerable morphological rearrangement of the synaptic connections between photoreceptors and second order neurons. During light adaptation, the terminal dendrites of horizontal cells (HCs) form numerous finger-like protrusions, so called spinules. The number of spinules is decreased during dark adaptation, and the retraction of spinules has been characterized as a CaMK II -dependent process but the role of calmodulin in this process remained elusive because it could not be localized to spinules.

Caldendrin, a novel neuron-specific Ca^{2+} -binding protein, is a member of a family of calmodulin-like proteins and highly abundant in brain. The protein was formerly called calp (calmodulin-like protein), indicating its similarity to calmodulin. Characterization of caldendrin revealed isoforms of 33 kDa and 36 kDa. The 33-kDa isoform is partly found in soluble fractions and the 36-kDa polypeptide almost exclusively in the aqueous and detergent-insoluble skeletal pellet. Caldendrin is present only in the somatodendritic compartment of neurons and never associated with axonal processes.

We hypothesised that caldendrin might be involved in the spinule retraction process and aimed to localize its distribution within the outer plexiform layer (OPL). Caldendrin expression patterns in the carp retina were analysed by means of immunoblotting, immunohistochemistry and immunoelectron microscopy. Polyclonal anti-caldendrin antibodies and monoclonal anti-CaMKII antibodies were used with standard protocols.

Immunoblot analysis of carp retinal samples, probed with anti-caldendrin antibodies, revealed a 36 kDa band in the membrane and a very weak band in the cytosolic fraction. A 33 kDa component was found in the cytosolic but not in the membrane fraction. Confocal microscopy of tangential and radial sections of the retina, revealed strong, punctuate labeling of caldendrin immunoreactivity within the OPL. In double labeling experiments with anti-caldendrin and anti-CaMKII, immunoreactivity was located next to each other in the same cone pedicle, at the site where HC terminals invaginate. Immunoelectron microscopy of light adapted carp retinas revealed strong caldendrin immunoreactivity in the dendritic HC terminals invaginating the cone pedicles. The label was concentrated in spinules and at sites adjacent to the base of the synaptic ribbon. In dark adapted retinas, spinules were retracted, and labeling was prominent along the membrane of the HC dendritic terminals. Label was also present in dendrites of HCs and bipolar cells invaginating rod spherules. CaMKII immunoreactivity was restricted to HC dendritic terminals invaginating cone pedicles, and was most prominent in spinules of light adapted retinas and at the membranes of HC dendritic terminals of dark adapted retinas. This pattern was identical to the one found for caldendrin immunoreactivity, confirming the close association of the two markers derived from the confocal analysis.

These data demonstrate an identical localization for caldendrin and CaMKII in HC dendrites, invaginating cone pedicles. They are in line with the assumption that caldendrin is the calcium-binding protein involved in CaMKII-dependent retraction of HC spinules during dark adaptation.

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Evaluation of the mouse „dark – flash“ electroretinogram (ERG) for further characterization of wild-type and knockout mice. 520

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ERGs are often used to functionally characterise specific deficits of knockout mice. Light flashes are applied during constant darkness (rod ERG) or superimposed onto constant background illumination (cone ERG) to characterize rod and cone pathways, respectively. However, those retinal neurons that are part of the off pathway respond preferentially to darkening. In many, especially cold blooded species, this is thought to be reflected in a distinct d-wave of the ERG, which occurs in response to cessation of prolonged light stimuli.The mouse, however, does not produce a clearly distinct d-wave in its ERG.

In this study the ERG responses to darkening from various light adapted levels were characterised using a “dark-flash” stimulus paradigm. ERGs were recorded from several strains of mice, including knock-out strains, using standard recording procedures. Stimulation was as follows: Eyes were light adapted for several minutes at different illumination levels. After adaptation, light was switched off (“dark-flash”) for short periods of times, whose duration was varied, and the resulting responses were analysed.

Responses to dark-flashes consisted of small positive or negative waves during darkness (dark-response) and a more prominent positive wave after switching back to the adapting light (light response). In wild type mice, the dark response shape depended on the previous adapting light intensity. It changed from positive to negative to oscillating with increasing light intensity. Oscillations were reduced in CX36 -/- mutant mice. The light response amplitude generally increased with adapting light intensity and resembled the b-wave from the “normal” mouse ERG. In CX36 -/- and BSN-/- mutant mice amplitudes were reduced compared to their respective wild-type littermates, as expected from previous studies (1, 2). At a given adapting light intensity the amplitude of the response increased approximately exponentially with dark flash duration, reflecting recovery from the previous adaptation. Time constants of this exponential recovery increased with adapting light intensity.

Dark-flash ERGs of wild-type and knock-out mice follow fixed response patterns. Therefore, dark-flash ERGs might allow further characterisation of functional deficits in genetically manipulated animals.

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521 Temporal structure of retinal ganglion light responses improves stimulus estimation.

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Light responses of many retinal ganglion cells display distinct spiking patterns. In the turtle, these patterns consists of 2-5 short bursts, occurring between 50 and 150 ms after light onset. These patterns have been reported as oscillatory or rhythmic bursting, both in mammalian and non-mammalian retinae (1,2).

Basic features of temporally changing stimuli can hardly be estimated from spike trains of single ganglion cells, using artificial neural networks, linear decoders or discriminant analysis (3). Estimation improves when the procedure uses those parts of the spike trains which are temporally locked to stimulus changes (4). This includes latency differences and initial spike rate into the estimation process (3, 4). Here we studied the relative contribution to feature estimation of this rhythmic bursting in the light responses of retinal ganglion cells.

Extracellular recording was done with a 100 channel multi-electrode system in isolated turtle (*Pseudemys scripta*) retinae. Ganglion cell activity was amplified, digitized and stored for offline analysis. Retinae were stimulated with light flashes of various intensities covering a 3 log units range.

The temporal structure of the spike responses changed according to intensity and position of the stimulus. Series of flashes with increasing light intensity evoked in many cells a typical spike pattern series. Latency of the first burst decreased, as expected, with increasing intensity. Another characteristic burst between 90-110 ms, however, had a lesser decrease in latency. This implicates that the time interval between these two bursts, the interburst interval, increased with increasing light intensity. The potential contribution of this interburst interval to stimulus estimation was investigated using discriminant analysis. The spike rate during the entire light period, the latency of the first spike relative to stimulus onset, the interspike interval between the first two spikes, and the interburst interval in question were used as discriminant variables. The spike rate is thought to be the most important variable for this kind of feature estimation, but the latency of the first spike provides almost as much information (3). We found, in addition, that the latency became the most important variable if only ganglion cells with a clear burst pattern were used for analysis. Percentage correct discrimination increased three fold when based only on latency, and spike rate got a minor role. However, the use of latency of the first spike requires external information about each stimulus change. Opposed to this, the interburst interval provides an internal, relative latency change. Compared to the results based on latency, estimation based on interburst intervals re-

ached on the average 50 %. With the best cells 90% correct estimation (9% change level) was reached using only the interburst interval.

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Acute effect of reserpine on physiological responses of retinal neurons in turtle 522

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Reserpine has been used as a psychotropic agent in human medicine. It is known to deplete monoamine stores thus influencing extracellular monoamine levels. Its potential side effects in the retina have never been assessed under experimental conditions though it could have been helpful in alleviating those. The vertebrate retina posseses two major types of monoaminergic cells: the dopaminergic amacrine cell and the serotoninergic amacrine cell. Both are present in the turtle retina, and both belong to the wide-field amacrine cells which are known to exert modulatory effect over large retinal areas. In our study we impaled different retinal cells with sharp intracellular electrodes to study their response to acute reserpine treatment.

Posterior eyecup preparation of turtle were made and cut into 2-3 mm wide strips. These thick slices were pinned in a recording chamber, superfused in Ringer solution, and exposed to spots of white light (d=1.2 mm) for repeated 500 ms intervals. Reserpine and other drugs (D1 and D2 receptor antagonists) were added to the superfusate.

Our results indicate that many retinal cells are sensitive to reserpine. In most cases it abolishes responses to light on and off, or selectively reduces one or more component of the responses. However responses of several cells were unchanged. Most of the horizontal cells reacted to reserpine by depolarization and diminshed response amplitude. Since horizontal cells can be recorded from for an extended period of time, we tried to analyse the nature of their response to reserpine treatment. First, L-type horizontal cells were selected by spectral analyses and then the effect of the mixture of D1+D2 antagonists were studied. Since the preparation was in a moderately light-adapted state, dopamine levels must have been high in the extracellular space. Horizontal cells reacted with a fast hyperpolarization to light. The antagonist mixture slowed the response of the cell and slightly depolarized the membrane potential but did not change the response amlitude significantly. Combined application of D1+D2 receptor antagonists and reserpine slowed the response and caused significant depolarization and response amplitude reduction. We suppose that the latter two changes are due to the serotonin 2A receptors which by analogy with the mammalian retina may be localized on the photoreceptor cell

terminals. Experiments are under way to prove this hypothesis by using selective serotonin 2A receptor agonists and antagonists.

523 The role of the eye-microtremor in vision: Hyperacuity and signal detection

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Involuntary eye-micromovements constitute a persistent source of noise for the visual system. While microsaccades and slow drift movements are important to prevent fading of the neural image in the retina, the role of the ocular microtremor (OMT) is still unclear. The OMT in humans consists of random movements with a mean frequency of about 80Hz (Bolger et al., 1999, Spauschus et al., 1999). For the amplitude of the OMT the experimental data is conflicting, ranging from as low as 6 sec of arc up to one minute of arc. We used a biological realistic model of the vertebrate retina (Hennig et al., 2002) to study the influence of the OMT on the responses of retinal neurons. To this end the model was adjusted to match anatomical and physiological properties of the primate retina. Here we report two findings of this model study. First, the effect of the OMT on the detection of a vernier stimulus was studied, as it has been suggested earlier hat eye-movements could contribute to hyperacuity (Averil et al., 1925, Marshall and Talbot, 1942). Our results suggest that the OMT improves hyperacuity in the peripheral retina, where the separation on the receptors is relatively large. In the central retina however, the blurring due to the optics of the eye prevent a strong effect of the OMT. Summarizing briefly, the movements lead to a greater number of receptors to be activated, which allows for a better integration and thus an enhancement of resolution at later stages. The second finding is that the OMT induces a stochastic resonance like response amplification already in retinal ganglion cells, which is a consequence of several nonlinearities in the retinal network. With a contrast-modulated stimulus, we demonstrate that this could improve the detection of weak stimuli.

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Zebrafish morpholino knockdowns with altered retinal morphology

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The zebrafish has become a major animal model for studies of developmental biology and genetics during the last decade. Beyond the use of mutagenesis to create mutant zebrafish, the recently developed morpholino method enables us to generate specific knockdowns, so called morphants, by blocking RNA-translation via the injection of specific morpholino-oligonucleotides into early developmental stages. The resulting larvae lack a specific protein and, thus, express a particular phenotype. Here, we document the morphological alterations in several retina specific zebrafish morphants.

Freshly fertilized zebrafish eggs were injected with the respective morpholino-oligonucleotides (MOs) near completion of the one-cell-stage. Beta-carotene-15,15'-oxygenase (*bcox*), cone rod homeobox (*crx*), and sine oculis homeobox homolog 3a (*six3a*) MOs were injected to induce the respective morphant geno-/phenotype in the treated animals. Retinas of morphant zebrafish larvae were examined at 2, 3, and 5 days post fertilization (dpf) by standard histological staining techniques. Distinct types of neurons were identified by immunocytochemistry and electron microscopy.

bcox: In comparison to wild-type (WT) siblings, the numbers of cones (lectin-labeling) and rods (rhodopsin-labeling) were strongly reduced in the *bcox*-morphant retina. The remaining cones were identified in a restricted area of the ventral retina. They expressed shortened cone outer segments (OS) and electron dense deposits were revealed between OS membrane discs when observed under electron microscopy. Furthermore, the electromicrographs unveiled increased numbers of phagosomes in close proximity to the remaining OS as well as numerous apoptotic cell nuclei in the outer nuclear layer of the *bcox*-morphant retina. *crx*: So far, comparison with WT retinas revealed slightly shortened OS and a degenerated lens in the *crx*-morphant retinas. *six3a*: Until now, we observed a degenerated lens and slightly altered morphology in the ganglion cell layer (especially in the 2dpf stages) and in the retinal marginal zone of the *six3a*-morphant retinas.

Our data show that the morpholino technique is a potent new method to create model systems for hereditary diseases with known gene sequence in the zebrafish visual system.

525 Connexin36-dependent retinal function in mice with specific rod or cone photoreceptor input

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Purpose To analyze the impact of the lack of connexin36 (Cx36) on retinal function. For this purpose, rod and cone photoreceptor input was separated by means of double mutant mice deficient of Cx36 plus either Rho (rod opsin knockout, cone specific) or Cnga3 (cone cyclic nucleotide-gated channel knockout, rod specific). Methods Double mutant mice were generated by cross-breeding $Cx36^{-/-}$ mice with either $Rho^{-/-}$ or $Cnga3^{-/-}$ animals. The resulting double knockouts in the F2 were identified by RT-PCR. Mice were studied by scotopic and photopic electroretinography (ERG) using previously reported protocols (Saszik et al., J Physiol 2002; 543.3: 899-916, and Seeliger et al., Nat Genet 2001; 29: 70-74). Results In comparison to wild type animals, Cx36-deficient mice show clear abnormalities in the ERG. For very low stimulus strengths, the negative scotopic threshold response was absent. The most dramatic change in the scotopic recordings was observed in the oscillatory potentials (OPs) riding on the b-wave, which were reduced at low stimulus strengths up to 10^{-2} cds/m², about normal in an intermediate range, and completely absent at the highest stimulus strengths above 3-10 cds/m². OPs were also strongly reduced in photopic ERG. If the input to the retina was limited to rods (i.e. in the double knockouts with the Cnga3 model), the scotopic results were largely comparable to those in a single $Cx36^{-/-}$ mouse, indicating that the loss of the OPs observed is cone-independent. In both rod and cone-specific recordings, the basal amplitudes of the b-wave subtracted by the OPs were within normal limits. Conclusions The connexin36 knockout model was found to have at least two functional phenotypes. At very low stimulus intensities, the missing coupling of the AII amacrine cells in the inner retina may lead to reduced OPs and a loss of the scotopic threshold response. At high intensities, the missing coupling, perhaps in outer retina, causes a dramatic change to the rod-driven OPs that occur in response to very strong stimuli.

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Effects of adenosine triphosphate (ATP) on the electroretinogram (ERG) of the chicken retina *in vitro*

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Background:

For decades, ATP has been known to function as a neurotransmitter. Meanwhile, two receptor subgroups for ATP were identified from neuronal tissue: the ionotropic P2X and the metabotropic (i.e. G-protein-coupled) P2Y. Until recent years, there was almost no knowlegde about P2 receptors in retinal cells. Just in the last years, research revealed that different subtypes of these receptors are expressed in the vertebrate retina also. In recent studies, P2X and P2Y receptors were localized in different cell types throughout the retina. Thus, changes in the extracellular level of ATP should possibly influence the electrophysiological responses of the retina.

Methods:

From an enucleated chick eye a circular piece of the retina including retinal pigment epithelium (RPE) and choroid was transferred into a modified Ussing chamber. The upper/retinal and the lower/choroidal side of the preparation were electrically isolated and separately superfused with an adapted Ringer's solution. At intervals of 51 s, the retina *in vitro* was stimulated for 4 s by diffuse light (~8 x 10^{-6} W/cm^2) from a halogen lamp. ATP was applied for 0.7 h in two sets of experiments at concentrations of 10^{-3} M or 10^{-4} M respectively.

The following electrical potentials, light evoked as well as standing potentials (SP), were recorded before, during, and after the treatments: The transtissue potential (TTP, representing the ERG) was measured between two bath electrodes, one on each side of the chamber. Via a microelectrode positioned in the outer retina above the subretinal space, it was possible to separate the TTP into two constituents: the transretinal (TRP) and the transepithelial potential (TEP).

Results:

Within minutes after starting the treatment of the chicken retina with ATP, pronounced changes were observed in the recorded potentials at both concentrations. Already after 5 min, the amplitudes of the fast components of the ERG (i.e., a-, b-, and d-wave) were stongly reduced. The slow c-wave showed the opposite effect. At the latest after 15 min, the amplitude had increased in comparison to the control values. As proven by the light evoked amplitude of the TEP, this effect originated from the outer retina-RPE complex. In addition, the transtissue SP raised after the onset of the treatment due to a lasting elevation of the transepithelial SP. The transretinal SP displayed a rapid but only transient increase. After the ATP treatment, there was an almost complete recovery of all signals to the level of the control recordings.

Discussion:

It is likely that two types of response to ATP can be distinguished. The effect on the TEP (i. e. the raise of the c-wave) is possibly mediated by metabotropic P2Y receptors

in the outer retina or in the RPE, as it develops slowly. In contrast, the immediate changes of the fast components might be caused by the ionotropic P2X receptor subgroup in the inner retina.

527 How owls learn to see depth: Motion parallax inducing head movements as a function of age.

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Adult barn owls are able to generate a sense of depth by mimicking over time the viewing geometry otherwise obtained through spatial differences between the images seen by the two eyes simultaneously, called retinal binocular disparities (van der Willigen, Frost and Wagner, 2002, Depth generalization from stereo to motion parallax. J Comp Physiol A).

In other words, relative-motion-based depth perception (motion-parallax), that is formally equivalent to disparity-based depth perception (stereopsis), occurs when the head is moved from side-to-side through the distance that separates the eyes (interocular-distance). This type of behaviour is called peering, and could be used to determine viewing distance: the unique distance at which disparity and relative-motion specify equivalent depth differences.

A functional explanation for the finding that adult barn owls process relative-motion and disparity in an equivalent manner to determine depth differences might be that peering is used to scale binocular information during development. The rationale behind this hypothesis goes back to the observation that juvenile barn owls spend a considerable amount of time with peering during which the interocular distance increases by a factor 2.5.

The present study was designed to assess whether or not patterns of peering behaviour in juvenile barn owls (*Tyto alba*) change as a function of age. Our data (N = 8 owls) show that early in life the amount of time spend with peering increases with a factor 3.5. Most notably, after approximately 40 days of age peering behaviour reaches its maximum and becomes less intensified hereafter. Starting from the age of 25 days the averaged peering amplitude corresponded to the change in interocular distance of the young owls.

These data support the hypothesis that peering movements could be used to regulate the alignment of stereopsis with the full metric properties of the visual scene. It is therefore of some interest to determine whether the onset of disparity-based depth perception coincides with, or is preceded by, the ability to measure distance based on peering behaviour alone.

Investigating phosphene elicitation with the paired-pulse paradigm

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Background: Intracortical excitability can be investigated using the paired-pulse paradigm consisting of a subthreshold conditioning stimulus (CS) followed by a suprathreshold test stimulus (TS) (Kujirai et al., 1993). In the motor cortex, short interstimulus intervals (ISI) evoke an inhibition while at longer ISI facilitation occurs. These effects seem to be mediated by activation of inhibitory interneurons and facilitatory influences between I waves respectively (Ziemann et al., 1998). Similar effects have also been reported for the parietal cortex (Oliveri et al., 2000). We investigated whether comparable inhibitory or facilitatory effects could be observed in phosphene elicitation using the paired-pulse TMS technique over the occipital cortex in normal volunteers. In case of a different underlying mechanism in the visual cortex, different effects of the paired pulses would be expected.

Methods: We examined phosphene perception to focal TMS with the paired-pulse paradigm in ten normal volunteers using two Magstim 200 stimulators connected via a Bistim module. We systematically varied ISI (2ms, 3ms, 8ms, 12ms), the intensity of CS (100%, 90%, 80%, 70%, 60% of phosphene threshold (PT)) and the intensity of TS (100%, 110% of PT). In another set of experiments, we explored different coil orientations (coil handle pointing upwards, horizontally to the left, horizontally to the right) while subjects reported phosphene perception.

Results: Facilitation occurred for all ISI ($p<0.001$) at CS 90% and TS 100% of PT. No inhibition could be observed. There were no systematic differences between different ISI. With CS intensity decreasing to 80% of PT or less, facilitation disappeared.

Conclusion: We found facilitation independently of ISI. Our results indicate that the mechanisms underlying phosphene induction in the visual cortex are different from those underlying MEPs for the motor cortex and do probably not rely on interneurons.

Ghosting and Aliasing Artifacts in Apparent Motion Displays Eliminated with Motion Blur

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Computer monitors and other apparent motion displays often produce artifacts such as ghosting and aliasing when displaying moving scenes. Although only low frame rates are necessary to produce the perception of motion, even frame rates substantially faster than our flicker fusion frequency can produce artifacts. Ghosting, one of these artifacts, is the perception of multiple simultaneous copies of spatially offset image features. Aliasing, another artifact, can result in perceived direction reversal of moving periodic patterns such as the classic wagonwheel illusion.

A model for apparent motion displays and early visual processing was developed and tested using psychophysical experiments on human observers viewing either a true motion stimulus (rotating drum) or a computer display. Tests with the computer display were performed both with and without artificial motion blur predicted to eliminate artifacts. In an object recognition task, observers were asked to identify which of two alternative face-sketches moved past an aperture. In a motion detection task, observers were asked which of two-alternative sequences of (2 or more) frames contained motion.

Our model shows that ghosting results from the slow time course of photoreceptor response. The 'neural image' of an un-blurred feature presented on one frame persists into subsequent frames where it reappears at new locations. Without motion blur, object recognition was possible in animations at velocities far beyond those where performance fell to random levels for true motion due to artificially high spatial detail. Un-aliased motion energy in animated scenes is not affected by artificial motion blur and we found that performance in the motion detection task was not significantly affected by its use.

In conclusion, artificial motion blur eliminates ghosting and aliasing on apparent motion displays to produce more realistic simulation of motion. Eye movements may confound this solution, so complete elimination of such artifacts requires frame rates over 1000 Hz or implementation of real-time motion blur based on eye tracking.

530 Slow feature analysis yields a rich repertoire of complex-cell properties

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In this work, we investigate slowness as a coding principle for the primary visual cortex (V1). The slowness principle can be illustrated as follows: the input signals to the cortex originate from the sensory cells by raw, local measurements of the environment. Such measurements are extremely sensitive to small changes in the state of both the environment and the observer, and vary thus on a timescale faster than that of the environment itself. In the specific case of V1, the sources of the visual input are objects and lights and their position in space. Single receptors on the retina measure light intensities at a given position, an information which is very sensitive even to small shifts or rotations of textured objects or the direction of gaze. Our hypothesis is that the cortex extracts slow signals out of its fast varying input in order to reconstruct information about the environment. This is equivalent to optimizing the neurons to have an output that varies in time as little as possible. To test this hypothesis, we considered video sequences derived from natural images and, by applying slow feature analysis (SFA), determined units (functions) that extract the most slowly-varying signals from the sequences. We analyzed the units by computing their optimal stimuli (i.e. the stimulus that maximally excites or inhibits a given unit) and by means of special test images that probe their response to a range of frequencies, orientations and phases as well as their end-inhibition behavior. We also performed experiments with drifting sinusoidal gratings to permit a direct comparison with experimental data in the literature. The analyzed units have many properties in common with complex cells in V1, including not only Gabor-like optimal stimuli

and phase-shift invariance but also direction selectivity, non-orthogonal inhibition, end-inhibition and side-inhibition. Our results show that a single unsupervised learning principle can account for such a rich repertoire of receptive field properties.

Receptive Field Plasticity in Area 17 Outside the Projection Zone of a Circumscribed Monocular Retinal Lesion. 531

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The spatial shift of 'ectopic' discharge fields (DFs), observed in area 17 (V1) neurons after retinal lesioning, may be a physiological correlate of the 'filling-in' effect (the underestimation or total unawareness of the 'missing' visual field) observed in humans with retinal scotomas. These individuals have also reported spatial distortions that extend well beyond the region of the visual field corresponding to their scotoma (see for review Dreher *et al*., 2001). In the present study we examined the possibility that the latter effect has a physiological correlate in mammalian primary visual cortex. For this purpose, eight week old kittens were anaesthetized (ketamine 20 mg/kg i.m.) and a 10-12 degree diameter region of the nasal retina in one eye was destroyed with an argon laser. 28-68 weeks later the animals were anaesthetized with gaseous mixture of N2O/O2 (67/33%) plus halothane (0.5 - 0.7 %), paralysed (gallamine triethiodide 7.5 mg/kg/h i.v.) and artificially ventilated. In lesioned cats the DFs of binocular area 17 neurons (recorded extracellularly) were delineated in both the LPZ and in the region outside the LPZ. As a control we delineated the binocular DFs of neurons recorded from regions of equivalent horizontal eccentricity in the striate cortices of normal non-lesioned cats. DF incongruity refers to the distance between the contra- and ipsilateral eye DFs of a binocular neurone when the *areae centrales* of both eyes are aligned. In the case of vertical incongruity, the cell population located outside the LPZ displayed a highly significant ($P < 0.0003$) directional bias relative to cells recorded from regions of equivalent horizontal eccentricity in normal non-lesioned cats. The response properties (e.g. the size of the DFs and the orientation, direction and velocity selectivities) of neurons recorded from outside the LPZ did not differ significantly depending on the eye to which the stimuli were presented (similar to neurons recorded from the LPZ; see Dreher *et al*., for review). The present data indicate therefore that a circumscribed monocular retinal lesion made during adolescence can induce topographic reorganisation not only in the LPZ of area V1 but also in the non-deafferented region surrounding the LPZ. Furthermore, in view of the stark similarities between the response properties of ectopic DFs and their non-lesioned eye counterparts it appears likely that the topographic reorganization in area 17 we have observed outside the LPZ is based principally on the strengthening of existing cortico-cortical connections. Congruent with this paradigm, post-mortem histology indicated that the retinal tissue surrounding the lesion was unperturbed.

532 Lateralized Neuronal Processing of Visual Information in "Pulvinar Inferior"

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The tectofugal pathway of the pigeon (Columba livia) is the functionally most important visual pathway for object analysis. Most of the ganglion cells of the pigeon´s retina project to the tectum, from where a prominent pathway ascends to the thalamic nucleus rotundus, a nucleus homologous to the inferior/caudal pulvinar of mammals. Although the optic nerve in birds crosses completely, visual information from the ipsilateral eye also reaches the nucleus rotundus by recrossing fibers, connecting the contralateral tectum opticum with the ipsilateral rotundus.

Recently, Schmidt and Bischof (2001) showed a convergence of contralateral and ipsilateral visual input on single neurons already at the level of rotundus. But they performed their recordings only on the right hemisphere.

To contribute to the question of possible asymmetries in the integration of visual information in n. rotundus we performed our recordings on both hemispheres and asked for the possible origin of the ipsi- and contralateral input to binocularly driven rotundal neurons.

We anesthetized our pigeons with urethane (20%, 0.1ml/100g) and mounted them in a head-holder. Stereotaxic coordinates for electrode penetrations were derived from an atlas of the pigeon (Karten and Hodos, 1967). Recordings were performed on both hemispheres. Light stimuli of 500ms duration were produced by two shutters and transferred to both eyes by oculars, which fit closely to the eye rim. Extracellular activity was recorded from single cells with glass-insulated platinum-iridium electrodes. Spikes were amplified, filtered, and stored for off-line cluster analysis.

We recorded responses of 142 rotundal neurons (72 neurons per hemisphere) to light-stimulation of the right, left and both eyes. Additionally, control recordings without stimulation had been performed. T-test comparisons with spontaneous activity classified neurons as binocularly or monocularly driven.

To investigate possible forebrain efferents on rotundal neurons we injected 100nl lidocaine into the right visual Wulst of some pigeons for temporally inactivating this brain region. Before, during and after this injection the responses of rotundal neurons were recorded during ipsilateral, contralateral and bilateral stimulation of the eyes.

Our results show, that information from both eyes converge onto single neurons already at the level of rotundus and proceed from there as mixed information to upper visual areas. There is an hemispheric asymmetry of integration. About 70% of neurons in the right rotundus integrate information from both eyes, but only about 30% of neurons in the left rotundus. Response latencies were much longer for the ipsilateral than the contralateral eye. This might be due to forebrain efferents, because there was a robust effect of Wulst blockade to the ipsilateral stimulation of binocularly driven rotundal neurons.

Our results might explain visual lateralization effects, as can be seen in object discrimination or in spatial tasks, on a very early visual level.

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γ-Frequency Fluctuations of the Membrane Potential and Response Selectivity in Cat Visual Cortical Neurons 533

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Fluctuations at frequencies of 25-70 Hz constitute an emergent property of cortical activity *in vivo*. These rapid, γ-range fluctuations are apparent in the local field potentials, spiking of cells and cell groups, as well as in the membrane potential of neurons. Presence of γ-band components in fluctuations of the membrane potential improves the precision and reliability of translation of the membrane depolarizations in trains of action potentials.

To investigate stimulus dependence of the γ-frequency fluctuations of the membrane potential, we have recorded intracellularly responses of cells in cat visual cortex to presentation of moving gratings. We have found γ-range fluctuations of the membrane potential in both simple and complex cells. The strength of the γ-frequency fluctuations correlated with the stimulus optimality. Furthermore, the amplitude of the γ-frequency fluctuations correlated with the phase of stimulus-imposed slow changes of the membrane potential.

The combination of these features makes cortical neurons capable to encode the slow changes in the visual world in a kind of amplitude modulation of the high frequency fluctuations. This assures reliable transformation of the membrane potential changes into spike responses without compromising the temporal resolution of visual information encoding in the low frequency range.

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The features of visual stimuli influence the orienting behavior in the frogs *Bombina orientalis* and *Discoglossus pictus* 534

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In amphibians, the gross categorization of prey objects during fast decisions for capture was recently reported in salamanders, in which different preferences for visual features were found. In this study, the orienting behavior of the frogs *Bombina orientalis* (N=12) and *Discoglossus pictus* (N=5) towards animated prey-dummies was studied using the same type of stimuli. Two out of eleven stimuli differing in contrast, size, velocity or movement patterns were moved in opposite directions on a computer screen. The orienting turns of the body were used to establish a rank order for the different stimuli in both species.

On average, *B. orientalis* responded most to the fast moving cricket, the large-sized cricket, the standard cricket, the still-image cricket, and the rectangle. The mean of responses to these five stimuli are close. There seems to be no specific rank order amongst them. Fewer responses occurred towards the slowly-moving cricket, the stepwise-moving rectangle, the locally-moving cricket and the stepwise-moving cricket. Again, the numbers for responses to these four stimuli were similar. The smallest numbers of responses were found at presentation of the small-sized and the contrast-reduced cricket.

The rank order was different in *D. pictus*. It mainly concerned the much lower responses towards the fast-moving cricket compared to *B. orientalis*. Another difference is the overall smaller number of responses compared to *B. orientalis*. In both species side preferences were not found for the group of animals (χ-square test). When frogs were tested singly, one *B. orientalis* and one *D. pictus* had a high preference for the left side.

The results of this study concerning *B. orientalis* correspond those of the above-mentioned study on the salamander (*Plethodon jordani*). In both species size and velocity are the dominant features to release prey capture. Shape and movement patterns are less important. Finally, the contrast-reduced cricket was among the stimuli with small numbers of responses in both species. In general, the data of *D. pictus* are comparable with those of *B. orientalis* and *P. jordani* except that velocity is less important in *D. pictus*.

However, statistical analysis of data is currently in process. It is open, whether the rank order will be exactly the same when using the maximum-likelihood method, and whether stimuli with comparable high numbers will be located in same clusters according to the complete-linkage method.

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535 Pulling at both ends: Attentional modulation of stimulus selectivity in macaque area MT

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Based on recent experiments a multiplicatory gain mechanism (MGM) of selective visual attention has been proposed which states that responses to attended objects are enhanced by a common gain. According to the MGM-model tuning curves of neurons are “pulled” in one direction. Due to the multiplicative nature of this process, the absolute firing rate increase is strongest for the response to the preferred stimulus and proportionally weaker for less preferred stimuli. However, a more powerful attentional shaping of neuronal tuning would be achieved, if tuning curves are pulled at two ends and in opposite directions. By pulling up the tuning curve’s maximum and pulling down its minimum, attention would lead to a genuine increase of stimulus selectivity.

We tested this hypothesis by recording neuronal activity from area MT in two macaque monkeys. The animals were engaged in a motion tracking task (adapted from Treue,S. &

Maunsell,J.H.R. (1996) Nature 382: 539ff.) that required them to attend to one out of two simultaneously presented moving bars. Activity during three subsequent motion cycles for preferred and anti-preferred directions was analysed.

As expected from the MGM-model, average neuronal activity was enhanced for attended motion in preferred direction. This increased activity level did not change over time. However, for motion in the non-preferred direction we found that 38% of all recorded neurons showed larger responses when attention was directed outside the Receptive Field. The fraction of cells showing this surprising behavior increased over time to 74% during the third motion cycle. The enhanced responsiveness for unattended non-preferred stimuli causes a significant reduction of directional selectivity during the second and third motion cycle as compared to the first for 28% and 51% of all units, respectively, resulting in a significantly reduced stimulus selectivity of the whole population. In a second experiment we increased task-difficulty and inter-stimulus competition. Effect magnitude was substantially increased with the same qualitative pattern of results: At the population level, stimulus selectivity was unchanged in the presence of attention, but decreased by 13%, 25% and 37% of all neurons during the three motion cycles without attention.

The present data show that selective attention can exert a substantial influence on neuronal stimulus selectivity. Without attention, stimulus selectivity suffers from a continuous degradation. Our findings imply that local feature contrasts of non-attended objects are obscured, thereby reducing saliency of stimuli in the absence of attention. This may explain why stimuli outside the focus of attention suffer from a reduced ability to attract attention and hence to be explicitly perceived.

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Population Analysis of Stimulus Representation in Rat Primary Visual Cortex **536**

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A timely question of systems neuroscience is how information about the sensory world is encoded in the distributed responses of a population of neurons. In this study we addressed this problem by analyzing how a single stimulus parameter, orientation, can be extracted from a population of neurons, recorded in rat primary visual cortex. Stimulus orientation was estimated from population activity with a Bayesian reconstruction technique, which has been shown to be superior to other population reconstruction techniques (Zhang et al., *J.Neurophys* (1999) 79, 1017-1044).

Recordings were done in area 17 of isoflurane-anesthetized Brown Norway and Long Evans rats. We used a multi-site recording technique based on micromachined silicon probes which allowed us to record from cells throughout cortical depth in a minimally invasive way. Visual stimuli consisted of whole screen black and white gratings with fixed spatial frequency, either moving with constant velocity or displayed statically in rapid succession. The latter kind of stimulation allowed us to characterize neural stimulus selectivity with a subspace reverse correlation technique (Ringach et al., *Nature* (1997) 387, 281-284). Bayesian analyis is based on the conditional probability estimate of neural responses to a given stimulus direction. Stimulus direction can be inferred from this distribution by an estimator. We found maximum-a-posteriori and mean estimator to yield similar results.

We have recorded from a total of 90 neurons so far. For each neuron we computed PSTHs and the directional tuning curve. A substantial proportion of all visually responsive cells were found to be orientation or direction tuned. Stimulus selectivity was found from the first responses on (~80ms after stimulus onset), as revealed by the subspace reverse correlation method.

Bayesian analysis often presupposes independent firing of cells and a Poisson firing statistics of individual neurons. We tested the validity of these assumptions. Almost all neurons showed bursty activity, and their trial to trial firing rate variability exceeded that expected for Poissonian firing. Thus, the Poissonian assumption could not be made for our data set! Many cell pairs showed significant synchronization as revealed by cross-correlation analysis. However, no indication of a statistical interdependence of *firing rates* between cells was found. Thus, while neurons can be synchronized at a time scale of several milliseconds, the assumption of independence of firing rates over several hundred milliseconds can still hold. Bayesian population analysis revealed that some neural subpopulations of 36 cells can yield an estimation accuracy sufficient for successful directional discrimination (accuracy of ±5°), while others only allow for an accuracy of ±45°. Discrimination accuracy improved with the duration of the response interval considered for up to 500ms after stimulus onset. Factors influencing the population discrimination accuracy are currently analyzed.

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537 Modulation of Striate Cortex Neurons by Attention in a Motion Tracking Task

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Using an elegant motion tracking paradigm, Treue and Maunsell (Nature, 382:539-541) demonstrated that the activity of neurons in macaque area MT is modulated by selective attention. Since area MT receives its main input from primary visual cortex (V1), we were interested to see whether effects of sustained selective attention visible in MT can also be seen at this first stage of cortical motion processing. Furthermore, according to the Biased Competition Hypothesis, the influence of attention on the activity of neurons is closely linked to the distance between attended and distracting stimuli. We used two

variations of the motion tracking task to assess the influence of spatial distance between attended and distracting stimulus on attentional modulation in V1.

The motion tracking task required the monkey to fixate a small spot in the center of the screen, while two bars moved back and forth along a given trajectory. The animals task was to detect the change in velocity of one bar (target) while ignoring a possible acceleration of the other bar (distractor). A spatial cue, shown shortly before onset of the bars, indicated the target in the following trial. In the first variation both stimuli were presented in opposite quadrants of the visual field, moving on parallel trajectories but opposite directions. In the second variation both stimuli where presented close to each other in the same visual quadrant, moving on orthogonal trajectories. Care was taken, that only one stimulus trajectory passed through each neurons receptive field. Recorded data were separated into single and multi unit activity. PSTHs were calculated and further analysis was performed on attentional indices (AI), calculated as the contrast of mean activities between attended and ignored condition, after subtracting spontaneous activity.

Data analysis showed that activity in V1 neurons was significantly modulated by selective attention. Otherwise identical stimuli evoked higher neural responses if they served as the target of attention. The modulation of activity was higher for target and distractor close to each other as compared to distant stimuli. This result confirms the corresponding prediction of the Biased Competition Hypothesis. Analysis of the electrodes' penetration depth profiles and electrophysiological response properties showed higher attentional modulation in the supragranular layers as compared to infragranular layers. These results provide further evidence, that the response of neurons in striate cortex can be modulated by selective attention, given a sufficient demand on attentional processing.

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Attention modulates synchronous activity in monkey area V4 in a shape tracking task 538

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Successful behavior in a natural environment requires selection of complex behaviorally relevant stimuli. This selection and subsequent processing of stimuli - i.e. integration of many different features into a complex shape in the presence of other shapes - depends strongly on attention. Oscillatory neuronal synchrony has been proposed as a mechanism underlying selective attention (Niebur,E. and Koch,C. (1994) J Comp Neurosci 1, 141-158) as well as a mechanism to integrate different features into a coherent percept (Singer,W. (1999) Neuron 24, 49-65).

To study whether oscillatory neuronal synchrony may serve as a mechanism of attention, we introduced a new shape tracking task which requires sustained attention to one of two objects morphing through a sequence of different shapes. During this sequence the

reoccurrence of an initially presented sample had to be indicated for the attended object. As a reliable measure for oscillatory synchrony field potentials (FPs) were recorded with a chronically implanted array of epidural electrodes covering area V4. To quantify synchronous oscillatory activity the current source density was computed based on the recorded FPs and analyzed in the time-frequency domain by convolution with complex Morlet's wavelets.

Analysis of the frequency content of the stimulus-induced signals revealed a distinct synchronous oscillatory activity pattern within the γ-band peaking between 50 and 60 Hz which is not phase locked to the stimulus. The responding region within area V4 was confined to the retinotopic representation of the visual area covered by the stimulus as judged from a previously constructed retinotopic map. Attention was found to increase oscillatory synchrony strongly (up to 80%) within the population of neurons which represent the stimulus. The strength of the attention-dependent oscillatory activity was found to be higher in phases of the task during which behaviorally relevant information of the stimulus had to be processed. Furthermore, it was shown that behavioral errors indicating a misdirection of attention lead to a predictable effect on the strength of synchronous oscillations. If a stimulus was erroneously attended, a clear increase of oscillatory activity was observed, whereas if a stimulus was erroneously not attended oscillatory activity decreased. Oscillatory activity was less affected by errors which are not expected to be directly related to attentional mechanisms. A control experiment showed that the modulation of synchronous oscillatory activity was not related to memory aspects of the task.

These results indicate that oscillatory neuronal synchrony is well suited as a mechanism of attention. We suggest that the strong effects observed for the new shape tracking paradigm may indicate that oscillatory synchrony can serve both functions of attention: stimulus selection and construction of structural descriptions for objects with a complex shape.

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539 Influence of attention on synchronized activity in macaque area MT

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Selective visual attention serves to select behaviorally relevant objects from complex visual scenes. Neurophysiological and imaging studies have demonstrated that attention modulates firing rates even in early visual areas. However, it has been suggested that attentional selection might be supported by stronger synchronization of the neural assembly representing the attended stimulus.

To test this hypothesis, we carried out multi-electrode recordings in macaque area MT under different attentional conditions. In a motion tracking task (adapted from Treue & Maunsell, 1996: Nature 382: 539ff.), two monkeys were required to attend extra-

foveally to one of two simultaneously presented moving bars, while fixating a small spot in the center of the screen. The monkeys' task was to signal a small accelaration of one of the bars, while ignoring accelaration events of the distracter bar. Behavioral relevance was randomly assigned to one of the bars and indicated to the animal by a spatial cue prior to trial start. Thus, the task allowed for identical visual stimulation under two attention conditions.

Multiple single- und multi-unit activity was recorded in area MT. Pairs of neurons were selected for recording when they showed similar direction preferences and sufficient overlap of receptive fields (RF's). In each of two experiments one bar was located in the region of overlap of the RF's and moved in the cells' preferred and anti-preferred direction. The second bar was located either in the opposite hemifield (Exp.1) or next to, but still outside the RF's moving along a an orthogonal trajectory (Exp.2).

Data were analyzed by computing crosscorrelograms and corresponding power spectra. In both experiments an attention-dependent increase of oscillatory synchronized activity was observed. When the bar over the RF's served as the target stimulus, the recorded cell pairs showed a higher degree of synchronization with precise phase alignment and preserved this pattern over the whole time of stimulation, i.e. for several seconds. In contrast, with attention directed away from the RF's we observed a smaller amplitude of the synchronization peak and strong fluctuations of its phase alignment. Significant oscillatory activity in the γ band was frequently observed for the attended condition, but was nearly absent in the non-attended condition. Similarly, an analysis of the spectral power of all recorded cell pairs demonstrated a general increase in γ power with attention. Both experiments yielded qualitatively similar results, but effects were stronger with higher attentional demand and larger inter-stimulus competition in the second experiment. Our results support the idea that changes of synchronized oscillatory activity form a mechanism of attentional selection.

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The *ticklish* spots of cortical orientation maps **540**

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Neurons in primary visual cortex preferentially respond to visual stimuli of a particular orientation. Across cortex, these preferred orientations are arranged in complex two-dimensional patterns, called orientation maps, and vary in a smooth manner except at characteristic singularities, called pinwheel centers. Accumulating evidence suggests that orientation maps can be shaped by neuronal activity through activity dependent mechanisms of synaptic plasticity. It has been argued that orientation maps in the visual cortex of the adult brain are in one of several possible states of dynamic equilibrium of a cortical learning dynamics [1]. This theory predicts that orientation maps (1) can be perturbed by experimentally applied activity patterns and (2) should subsequently rear-

range to either return to the previous or evolve to a different equilibrium configuration. Recent experiments in the visual cortex of adult cats have indeed shown that changes of the entire orientation map layout can be induced by intracortical microstimulation [2]. Furthermore, Godde and coworkers demonstrated that only in some instances did the map return to the previous configuration subsequent to the experimentally induced rearrangement. Whereas in other instances, the map spontaneously rearranged towards a new pattern significantly different from the initial map.

Here we present a minimalistic mathematical model for such perturbation induced rearrangements of cortical orientation maps. We constructed a generalized Swift-Hohenberg equation including nonlocal interactions that account for long-range synaptic interactions present in the cortex. The nonlocal terms are crucial for the stability of aperiodic patterns. To simplify the mathematical analysis, we considered a reduced 1D equation that exhibits all essential features of a cortical learning dynamics, which are (1) the existence of multiple stable aperiodic orientation maps (2) occurrence of pinwheel-like orientation defects. We numerically investigated the dynamics of such aperiodic map under a local perturbation. We show that the magnitude of changes induced by local stimulation can be predicted from the spectrum and eigenfunctions of the linearized equation. We furthermore show that the susceptibility of aperiodic orientation patterns to local stimulation strongly depends on the location of stimulation within the map. Most importantly, we demonstrate that the 'ticklish spots' of the map, i.e. locations of highest susceptibility to stimulation induced changes where identical to the locations of the maps pinwheel defects. Supported by the Volkswagen Stiftung and the Max-Planck-Gesellschaft.

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541 Attention controls spatial distortions in visual short-term memory

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Experiments show that human subjects make systematic errors when trying to remember the exact position of a briefly presented target dot in the presence of visual landmarks: targets are reproduced too far away from the landmarks and from the midpoints between landmarks (Werner & Diedrichsen, 2002). At the same time, it has been shown that the perceived position of a briefly flashed target is biased away from the focus of visual attention (Suzuki & Cavanagh, 1997).

In two experiments, we investigated the influence of visual attention on the pattern of spatial memory distortions. In Experiment 1, we compared conditions where the mouse cursor started either from a predictable position at the midpoint between two landmarks or from a random position. We observed larger distortion from the midpoint if this served as the starting point of the cursor so that it was able to bind attention. In Experi-

ment 2, we manipulated visual-spatial attention to the landmarks by having the landmarks jump to a new location during the retention interval in half of the trials. Groups of participants were instructed to reproduce the target either relative to the landmarks (so that the landmarks had to be attended) or to the absolute screen position (so that the landmarks had to be ignored). Distortions from landmarks were only found when landmarks had to be attended, not when they had to be ignored.

We present a stochastic neural-network model of activation in topographical memory maps to account for spatial distortions in visual short-term memory (Trommershäuser & Schmidt, in prep.). We assume that landmarks, midpoints, and connecting lines form a reference grid which is represented by activity in a memory map. However, we assume that the joint representation of reference grid and target location is a subadditive combination of the two single representations. Furthermore, the system is sensitive only to dynamic changes in activation beyond the static representation of the reference grid. Target representations are biased away from the major elements of the reference grid because the effects of subadditivity are larger there. We conclude that spatial short-term memory distortions could be explained by assuming that multi-landmark displays are explored by attention in a nonrandom fashion.

Neural mechanisms underlying the attenuation of target-distractor interference in visual search: Evidence from electromagnetic brain responses in humans

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Current source density analysis of magnetencephalographic and electroencephalographic brain responses was performed to investigate neural mechanisms that serve to attenuate target-distractor interference in vision. Observers performed on a search task in which the degree of feature overlap between target and distractor items was varied: Target and distractors shared featues on a single (color) or on two feature dimensions (color and orientation, double interference condition). Our results show (1) that focusing attention onto the target item leads to a focal enhancement of neural activity. (2) A comparison between experimental conditions revealed that source activity in these ventral occipito-temporal areas was larger in the double than in the single interference condition. These findings suggest that the attenuation of distractor-interference in visual search is closely linked to enhanced of neural activity in ventral stream cortical areas.

543 Strabismus does not enhance the segregation of ocular dominance domains in cat area 18

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Strabismus is a common clinical condition in which the visual axes of the two eyes do not meet at the fixation point. While the effect of strabismus on the functional architecture of the primary visual cortex (area 17) has been extensively studied far less is known about experience-dependent plasticity in area 18. In area 17 of normally raised cats, most neurons are binocularly driven and their orientation preference is virtually identical for the two eyes. This match is essential for stereoscopic vision. In contrast, in area 17 of squinting cats, neurons are responsive almost exclusively to stimulation of either the left or the right eye, the segregation of thalamocortical afferents into alternating ocular dominance (OD) columns is enhanced and horizontal interactions between different OD-columns are severely reduced compared to normally raised animals (Löwel & Engelmann, 2002). Furthermore, optical imaging of intrinsic signals revealed clearly segregated OD-maps in area 17 of strabismic but not in normally raised cats (Löwel et al. 1998; Engelmann et al. 2002).

We used optical imaging of intrinsic signals to analyze experience-dependent plasticity of cortical maps in area 18 of 8 cats (aged 2 - 6 months) raised with a surgically induced divergent squint angle. Four normally raised animals served as control. Cats were visually stimulated (monocularly) with moving high contrast square-wave gratings of 4 different orientations (spatial frequency: 0.15 cyc/deg, velocity: 12 deg/s). The vascular pattern of the cortex was visualized at 540 ± 10 nm (green), cortical activity maps were visualized with the ORA 2001 System (Optical Imaging Inc.) at a wavelength of 707 ± 10 nm (red).

Unlike our previous observations in area 17, activity maps in area 18 of strabismic cats induced after stimulation of the left or the right eye (monocular iso-orientation domains) were much more similar than in area 17, rather looking like area 17-maps of normally raised animals. Therefore visualization of OD-domains was only weak and no obvious difference in the pattern of OD-domains in area 18 of strabismic and normally raised cats was observed. Further quantitative comparison of the area 18-maps revealed that pinwheel densities were not different for strabismic and normally raised animals.

Our results demonstrate that the alteration of visual input can have different consequences for different areas of the visual cortex: While the decorrelation of activity between the two eyes (as induced by strabismus) clearly enhances the segregation of OD-columns in cat area 17, area 18 does not show this effect although the percentage of binocularly driven neurons is as reduced as in area 17 (Cynader et al. 1984). Since area 18 is distinguished from area 17 by a prominent Y-cell input, larger thalamocortical afferent arbors and larger receptive fields these factors are likely candidates for explaining the observed differences in the susceptibility to strabismus-induced plasticity between the two areas. Another possible explanation is that the substantial binocular interactions that have been observed in area 18 of strabismic cats (Cynader et al. 1984) manifest themselves in the reduced contrast on the optical OD-maps.

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Categorical colour coding in goldfish

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Purpose: It is already known, that goldfish are tetrachromats and can perceive a multitude of different colours. But how are these colours organized? Are colours perceived and memorized absolutely or are they combined in larger groups, the so-called categories (which are for example known in humans, apes and pigeons)? To examine the subjective perception of colour by the goldfish Carassius auratus in the range of 400 nm- 700 nm and to clarify the existence and crossover points of categories, behaviour-physiological experiments are performed. Methods: Naïve goldfish are trained with food reinforcement alternatively on two different lights, each of a certain wavelength (60 to 90 nm apart). Afterwards they pass through two different tests. 1.) The generalization test, which was already used to determine categories in pigeons (BLOUGH, 1961) and also in goldfish (GOLDMANN et al., 1991), determines the choice frequency of each of the trained or test stimuli, which are shown in comparison to a dark test field, during 2 minutes. 2.) In the transfer test two lights of certain wavelengths are shown simultaneously and 100 choices of the fish are counted. The test stimuli are chosen as to have the same perceptual distance to the trained stimuli (adjusted according to wavelength discrimination in the goldfish, NEUMEYER, 1986). As a control, a wavelength discrimination test is accomplished, in which one of the trained stimuli is shown in direct comparison to each test stimulus. In a further attempt fish are trained on six similar wavelengths, covering about 40 – 95 nm of the wavelength spectrum. Then, the generalization test was done for each of these wavelengths. Results: The results of the generalization test are nearly the same for the various training stimuli used. The choice frequency values decrease with increasing distance to the training stimuli. They show no remarkable differences or asymmetry in their progression. Most of the transfer test constellations reveal the same choice frequency (about 50%), which would be expected relying to the same perceptual distance from the training stimuli. However, in two of the tested combinations significant preferences for one wavelength occurred. The generalization results of the fish which where trained on the six wavelengths showed different choice frequencies on these training stimuli, though the training procedures were all the same. Conclusion: The results of the generalization test allow no general statement about possible categories and their borders. Rather they reflect the properties of wavelength discrimination. A direct comparison to the results of wavelength discrimination supports this conclusion. Though the difference in the choice frequencies in two transfer tests and also in the generalization test with the six training stimuli may be a cue of possible categories, clear evidence is missing.

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545 Experiments on visual perception in goldfish (*Carassius auratus*): What is more important - color or shape?

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Purpose: Honeybees show a clear preference of color over shape, when both attributes are combined in one figure. In this study corresponding experiments were done with goldfish.

It is known that many cyprinid fishes have a highly developed color vision. Goldfish possess a tetrachromatic color vision with four cone types with maximal sensitivities at about 356 nm, 447 ± 7.7 nm, 537 ± 4.7 nm and 623 ± 6.9 nm. Wavelength discrimination, color constancy and simultaneous color contrast have been investigated. Earlier experiments have shown the capability of visual discrimination of shape by cyprinid fishes. The fish seem to discriminate the shapes in terms of differences at the base or top line of the figure or the position of the apex.

Methods: Two goldfish were kept separately in tanks (40 x 25 x 25 cm). Two holes (8 cm diameter) of a 'feeding plate' (25 x 23 cm) served as test fields, on which two figures were shown using slide projectors. Food reward was given at the correct test field.

In the first experiments the fish were trained on a red triangle, a green square was given for comparison. In the second experimental series a black triangle and a black square were used, both only shown as contours. Transfer tests were performed in which the figures differed from the training situation in color and/or shape.

Results: In the first experiments there was no clear preference for color or shape. No preference at all was found with test fields containing a new attribute (for example both yellow or both circles) or with rotated shapes. In general, the fish seemed to prefer slightly the shape of the training field, priority of color was found in one test only.

In the second test series the training fields differed only in shape. In this case the choice frequency for the training shape was always higher than in the first experimental series. There was no preference for a certain color. But the two goldfish seemed to discriminate the shapes in other terms. One fish choose the triangle independent of the orientation, the other one choose always the figure with apex up.

Conclusion: In contrast to the honeybees, goldfish do not show a clear preference of color over shape. The tests indicated that discrimination took place in terms of shape rather than in terms of color. But the preference for the shape is not always clear. When the test fields contained a new attribute (color or shape) there was a complete loss of the

preference. This seems to indicate that the goldfish have learned both attributes of the figure as a whole.

Contrast-dependent motion detection in the zebrafish (*Danio rerio*): A comparison of the mutant "Fading Vision" with the wild type

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Purpose: Perception of motion is a basic property of visual systems. Using the optomotor response in goldfish and zebrafish, a dominance of the L-cone type and "colour blindness" in motion vision was found (Schaerer & Neumeyer, 1996; Krauss, 2001). In preliminary ex-periments with the zebrafish mutant "Fading Vision" we found that a higher intensity of white light was necessary to show the same response as the wildtype. To further analyse the visual system of the mutant we investigated the optomotor response with gratings of low contrast in comparison to the wildtype. Methods: The optomotore response was elicited using black and white striped cylinders of 10 different contrast steps. Two types of cylinders (diameter 14cm) were used: square-wave gratings and sine-wave gratings. The cylinders moved around the circular test tank (diameter 12cm) with a speed of 10 rotations/minute. The size of one light-dark cycle of the pattern was 4cm. Results: All fish showed an optomotor response to both types of cylinders. The response strength depended on contrast and pattern: with the sine-wave grating a three-times higher contrast was necessary than with the square-wave grating. This difference was the same in wildtype and mutant. However, the wildtype showed a three-times higher contrast sensitivity than the mutant. Furthermore, the slope of the functions differed, it was stepper with square-wave gratings than that with sine-wave gratings in the wild type, but not in the mutants. Conclusion: Contrast detection is better in gratings with high spatial frequencies (square-wave) than in sine-wave gratings. The overall poorer performance of the mutant "Fading Vision" maybe due to a shortening of the outer segments of the photoreceptors and defects in the pigment epithelium. The difference in slope may indicate defects in information processing. Maybe there are also other defects responsible.

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547 Spatial Frequency Channels in Striate Cortex of Awake Monkey: Receptive Field Properties and Mutual Signal Couplings

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Introduction and Goal. We are interested in the properties and neural mechanisms of spatial frequency (SF) channels in the visual system. Their psychophysical investigations in primates, including the adaptation to spatial frequency bands, revealed the existence of relatively few channels that are 1.) tuned to different frequency bands; 2.) selective for orientation; 3.) restricted in visual space. A spatial frequency channel might therefore, in first approximation, be viewed as a spatial Gabor-filter. Natural scenes are considerably composed of oriented contours containing locally often much broader spectral components than are passed by a single SF-channel. We therefore assume that a sharp luminance step, for example, is represented in the visual system by a superposition of several SF-channels with different passbands and a common central zero-crossing in their phases. We assume, in general, that local spectral properties occurring often correlated in visual space, will induce learning in the visual system, and thus will cause signal couplings among SF-channels in visual cortex. We investigated in monkey striate cortex whether such signal couplings are present among frequency channels of different properties, including their preferences to frequency, orientation, and spatial position.

Methods. Recordings of multiple unit activity were made at parafoveal representation in upper layers of striate cortex (V1) with an array of 16 microelectrodes in awake fixating macaque monkeys. At 3 different monitor distances we determined: positions and sizes of classical receptive fields (CRF) with randomly jumping spots; orientation and SF-tuning with Gabor patches of different orientations and SFs; correlations among SF-channels with stimulation by the contour and/or surface of a luminance-defined object.

Results. Our data show that response delay in local populations increases with increase in stimulus SF (1-10 cyc/°, 66-76 ms). While SF-preference had a range >3 octaves, CRF-size measured with jumping spots varied much less. Retinal CRF-size changed with stimulus distance in many cases but was nearly invariant in its average. However, response strength was modulated by distance showing different characteristics (*far, intermediate and near*). With activations by the luminance contour, often strong coupling (35-70 Hz) was present among neurons with different SFs prefering the contour's orientation and having overlapping CRFs.

Conclusions. We propose that the luminance step of a contour is coded by neurons synchronizing at high frequency and having overlapping CRFs such that the contour's profile is represented by the superposition of the CRFs. As we found neurons of any SF-preference within each class of distance modulation (*near, middle, far*) we assume that these populations are involved in coding objects in the fore-, middle-, and background of a scene.

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The encoding of saccadic eye movements within posterior parietal cortex

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Recent studies identified a number of functionally distinct subregions of posterior parietal cortex (PPC). Above all, activity within PPC is strongly modulated by attentional and visuomotor processes. We used fMRI (Siemens Magnetom Vision, 1.5 T, EPI, 30 slices, voxel size 3x3x4.4 mm3) to contrast the activation pattern during the execution of predictable and unpredictable saccades. In one case valid cues predicted the subsequent oculomotor target in the left or right visual field. In the other case neutral cues predicted only the upcoming target, but not its spatial location. We compared the resulting activation pattern with activity related to oscillatory saccades with different frequencies (1 and ½ Hz). FMRI scans were obtained and eye movements recorded (MR-Eyetracker) while eleven healthy subjects (mean age 22.6 years) performed separate blocks of the individual saccades. Data analysis was performed with BrainVoyager ($p<0.05$ corrected). Our results showed activation in largely overlapping networks with differing strength of activity and symmetry of involved areas. Predictable saccades led to an overall enhanced activity compared to unpredictable saccades. Furthermore, predictable and unpredictable saccades were dominated by activation within the right hemisphere, whereas oscillatory saccades were dominated by activation within the left hemisphere. Finally, the local activation maxima were generally located within posterior intraparietal sulcus (IPS). The predictable saccades were additionally represented in its anterior part.

The enhanced activity during the execution of predictable saccades was probably related to top-down processing and/or the preparation of the upcoming eye movement. The hemispheric difference could arise from a predominant role of the right PPC for shifting spatial attention and the left PPC for shifting temporal attention. The differential encoding of saccadic eye movements within IPS indicated that the PPC splits up into different modules related to the cognitive demands of a saccade.

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Neural dynamics of saccadic suppression

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Saccades displace the image of the environment on our retina, yet, we perceive the outer world as being stable. This perceptual stability has been attributed to a reduction of visual sensitivity around the time of a saccade. Psychophysical studies show that saccadic suppression mostly takes place in the dorsal pathway of the visual system and starts well before saccade onset. In our study we aimed at identifying a neural correlate of the dynamics of saccadic suppression in the macaque posterior parietal cortex.

Neuronal responses to brief visual stimuli were recorded in areas MT, MST, LIP, and VIP of two awake monkeys. Visual stimuli as well as saccade targets were back-projected onto a screen subtending the central 60° by 60° of the visual field. The animal received liquid rewards for correctly performing the saccade task. Saccades always had the same metric: along the horizontal meridian from 10° left to 10° right (in the second animal: from 10° right to 10° left). A brief visual stimulus (width: 10°, height: 60°, duration: 8 ms) was presented perisaccadically at one of six neighboring but non-overlapping locations. We did not want to rely on any hypothesis concerning when and to what extent a neuron's visual RF would shift relative to saccade onset. Instead, we stimulated, across different trials, the whole mapping range. We thus were sure that for each point in time one of our stimuli hit the most sensitive region within the RF.

For data analysis, alignment to stimulus onset allowed for defining a post-stimulus response window used for determining the average response elicited by the visual stimuli in the pre, peri, and post condition. Pre-stimuli occurred on average 550 ms before a saccade. Peri-stimulus occurrence ranged (in pseudo-randomized order) from about 200 ms before saccade onset to about 200 ms after saccade onset. Post-stimuli, again, occurred on average 550 ms after a saccade. A repeated measures ANOVA on ranks was employed to test for significant differences between responses in the pre, peri and post condition.

We show that a neural correlate of the saccadic suppression can be identified in motion sensitive areas MT, MST and VIP, but not area LIP. The time course of suppression of neural activity is strikingly similar to what has been found psychophysically. It starts 150 ms before the saccade, reaches a maximum at saccade onset, and, after a brief enhancement of neural responsiveness, it returns to normal 150ms after the saccade. Our findings imply that the visual system partially shuts down just before a saccade to avoid processing the spurious motion caused by the movement of the eye.

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550 Receptive Fields from Epi-retinal Recordings in Cats Give Hints for Optimizing Epi-retinal Implants for Blinds

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Introduction. Blinds with receptor degeneration can perceive localized phosphenes with epi-retinal focal electrical stimulation [1]. Recent work on the spatial resolution achievable with epi-retinal stimulation in anesthetized cats was done by assessing cortical activity distributions [2, 3]. They can be related to the position and form of phosphenes generated by epi-retinal implants. These cat experiments revealed a high variance in position and form of the cortical activation profiles that could not be explained by the retino-cortical mapping. Here we analyzed the reasons for this variance in order to improve the stimulation technique and hence, provide the chance for regular high-resolution perceptions with retina implants.

Methods. We used epi-retinal recordings because it is highly probable that the neurons whose signals are recorded by an electrode are mainly the same ones that can be activated by this electrode. We recorded local field potentials (LFP, 1-140 Hz) and spikes in anesthetized cats with multiple fiber-electrodes. The signals were separated by direct hardware- or offline-filtering from broad-band signals (2-10000 Hz). Visual classical receptive fields (vCRFs) were analyzed using a multi-focal visual stimulation approach.

Results. The positions and sizes of vCRFs were generally distinct for LFP and spikes (N=15). While the center positions of the vCRFs based on LFP closely resembled the retinal electrode positions and were concentric, spike-vCRFs from the same electrode were often multimodal, i.e., different spiketrains sorted from the same recording site had different vCRF-positions. They were often shifted distally but never proximally with respect to the optic disk. The connection lines between corresponding LFP- and spike-vCRFs were oriented parallel to the axonal fibers at this retinal recording location.

Discussion. The well localized LFP-vCRFs probably reflect mainly postsynaptic dendritic events at the parallel dendrites of bipolar cells and the somata of ganglion cells. This is probable because the graded potentials are well localized and correspond to the actual tip position of the recording electrode. The dislocations of spike-vCRFs, however, are indicative of the recording of spikes from axons originating more distally from the recording site with respect to the optic disk. Distally generated axon-spikes are detected when they pass the retinal recording site *en route* to the optic disk. Multi-modal spike-vCRFs are therefore indicative of recordings from several axons.

Conclusions. Our study of simultaneously recorded LFP- and spike-vCRFs for epi-retinal electrodes shows that one has to cope with ambiguous retinal and cortical activation, and hence with spatially ambiguous phosphenes with the presently developed epi-retinal implants. However, this may be avoided by a quasi-intra-retinal activation with protruding epi-retinal electrode tips that impinge on the retina.

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Population Activity in Cat Visual Cortex Evoked by Electrical Form and Motion Stimulation of the Retina 551

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An electronic retinal implant that may partially restore vision by bypassing damaged photoreceptors and electrically activate the remaining intact retinal neurons should not only evoke phosphenes but should also generate cortical representations of object forms and their motion. In a first approach to this, we analyzed visual cortex responses to basic electrical form and motion stimulation.

Methods: We inserted in anaesthetized cats seven hexagonally arranged fibre-electrodes through a small scleral incision and positioned them epi-retinally in the vicinity of the

area centralis. For the recording of cortical population activities (multi-unit spikes: MUA, local field potentials: LFP), we placed up to 16 fibre-electrodes in areas 17 and/or 18. Retinal and cortical electrodes were adjusted to corresponding sites. For electrical stimulation we used charge-balanced currents consisting of rectangular impulses of 200 µs duration and current amplitudes of 10-100 µA. Basic form stimuli were generated by the selective and synchronous activation of some of the 7 retinal electrodes. Movement stimuli were synthetized by consecutive presentations of neighbouring line stimuli. From cortical recordings we computed stimulus-related spatio-temporal activation profiles to estimate relations between (1.) retinal stimulation distance and spatial resolution, and (2.) stimulus velocity and spatio-temporal resolution. Influences by the retino-cortical pathway were assessed by comparing cortical activations evoked by form stimuli with those superimposed from corresponding single site stimulations. An analogous approach allowed us a coarse estimation of movement related neuronal interactions. In addition, we compared cortical responses to form and motion stimuli by a receptive-field-based backprojection of cortical activities.

Results: We confirmed our previous finding that electrical retina stimulation may yield a spatial resolution of 1-5 deg visual angle and a temporal resolution of about 20 ms for LFP and MUA. We found that the spatio-temporal cortical activation profiles are commonly related to retinal form and motion stimuli. Cortical activity analyses showed that for two-point form stimuli the strongest interaction occurred at distances of about 3° visual angle and that cortical responses to 'moving electrical bars' were better tuned for MUA than for LFP recordings. Projections of cortical activations to visual space were in appropriate relations to electrical form and motion stimulation of the retina.

Conclusions: Our results indicate that the stimulation of the retina with electrical form and motion stimuli can lead to geometrically related cortical activations. However, the selective activation of single cortical neurons with specific tuning properties by electrical retina stimulation and the cortical representation of long-term stimulation with retina implants should be addressed in future work.

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552 Slow Visual Feature Learning in a Recurrent Network of Spiking Neurons

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INTRODUCTION. Our visual system is able to recognize familiar objects independent of their size, position, lighting conditions and view. We want to analyze the underlying neural mechanisms by a biologically plausible model, including: 1.) temporal coherence of features in image sequences, 2.) combination of fast and slow forward projections, and 3.) fast feedback. The input stimuli consist of image sequences with objects moving continuously in 3D. It has been shown that networks, applying a trace learning rule [1,2] to similar stimuli, exhibit a high degree of invariance according to transformations present in the input images. Feedback connections, reaching further than intra-areal lateral projections, may play an important role in adding context interactions from

beyond the classical receptive field (CRF) [3]. We are also interested in the formation of CRF-properties, including their selectivity to orientation and spatial frequency, and the developing local coupling structure.

METHODS. We model the magno- and parvo-cellular pathways with respect to their temporal and spatial selectivities. Each pathway is made up of a hierarchy of topographic layers of spiking neurons [4]. Neurons within a single layer interact via local inhibitory and medium-range horizontal modulatory connections. Additionally, neurons interact via long range modulatory feedback connections from higher levels within and between the magno- and parvo-pathways. Due to the difference in their transmission speed feedback signals from the first can coincide with bottom-up activation from the latter. Feedforward connectivity is initially undetermined but modified through on-line memory trace learning [1]. Input signals to the model are generated by a 3D texture rendering engine, which allows free navigation through a 3D scene of textured objects with realistic lighting conditions.

DISCUSSION. Our network architecture is well supported by physiological findings. We argue that feedback signals from the magno-pathway, coinciding with bottom up activation in the slow parvo-pathway, play a significant role in the prediction of local feature arrangements and contrast enhancement and thus, will improve the signal-to-noise ratio compared to pure feedforward networks. Furthermore, this feedback arrangement allows integration of global context information on a much larger scale than possible with intra-areal connections. As a result of the learning procedure we predict the emergence of simple/complex-cell CRF-properties representing stimulus statistics. In particular, we will analyze the local coupling properties among neurons selective to different spatial frequency bands and compare them with those of monkey striate cortex recordings made in our group. Finally, we want to show that our model structure, learned from input with temporal coherence of image features, allows a more generalized representation of objects compared to learning from static images.

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Statistical analysis of the dynamics of intrinsic states in cat visual cortex

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We performed the analysis of the real-time optical imaging data from the primary visual cortex of anesthetized cats using methods of statistical learning theory. Optical imaging recordings using voltage sensitive dyes show that activity patterns with significant correlations with orientation preference maps arise spontaneously at least 20% of time. We found that ongoing activity is composed of a set of states, which switch dynamically. We applied a self-organized mapping algorithm (Kohonen, Self-organizing maps

[1997]) to determine the topology of these states and to study the dynamics in a genuinely independent way. The results confirm that states of spontaneous activity can be mapped onto a one-dimensional ring structure of templates similar to single condition orientation maps. Long epochs of smooth transitions between neighboring orientations were obviously present in the data. The duration of such sequences was much longer than typical temporal correlation times. We also calculated the typical speed of transition and typical times spent in certain states of spontaneous activity. We found a clear preference of ongoing activity for states correlated with single condition maps of cardinal orientations. The results show that the ongoing activity is tightly linked to its functional architecture.

554 Temporal patterns in neuronal ensemble data

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purpose to understand and identify "supraneuronal units" on the level of neuronal ensembles which might participate in distributed coding of visual information we simultaneously recorded the responses of small retinal ganglion cell populations. In the framework of the development of a visual prothesis we try to address the question: (i) what is processed, (ii) how does this processing occur by retinal ganglion cells? (iii)can "supraneuronal units" reliable identified? and (iv) what are general coding properties between retinal ganglion cell ensembles?

methods we use a 3-dimensional sharp tipped multi-electrode array to obtain extracellular recordings from retinal ganglion cells. our experiments employed superfused, flattened preparations of intact rabbit and human retinas. visual stimuli with simple physical properties (flashes, gratings, visual patterns, etc.) were used to probe the linear and nonlinear response characteristics of the random selected retinal ganglion cell populations. Instead of looking on single spikes we estimated the population responses and analyzed all identified units in this context.

results We applied different exploratory methods to detect *in vivo* intrinsic deterministic signals in the population responses on which we based the dissection of the recorded neuronal population into the identifiable, different temporal response "unit classes". By taking in account how which temporal responses classes are compositing a recorded population, we identified general, common coding properties of this classes. Additionally the detected deterministic intrinsic signals provide sufficient information to identify the physical properties of the visual stimulation from the estimated population responses.

conclusion we are presenting the application and implementation of several techniques to identify general, common features of *in vivo* neuronal ensemble data. Our results are fundamental for further chronic multichannel multi-unit recordings to address basic questions in modern vision and cognitive neuroscience.

ICMS induced plasticity in area 18 of adult cats: *Where have all the pinwheels gone?* 555

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So-called pinwheel (PW) centers are ubiquitous structural elements of orientation preference maps (OPMs). At these locations iso-orientation domains are arranged in a pinwheel-like fashion and neurons exhibiting the whole range of orientation preferences can be found in close vicinity. Locations of PWs in OPMs have been shown to be very stable for several hours in adult animals (1) and even for days and weeks during postnatal development (2). The question if PW patterns are genetically determined or at least fixed by wide-spread anatomical intracortical connections or if they are dynamically maintained and plastic is still under debate.

Using optical imaging, we recently demonstrated that widespread reorganizational changes of OPMs can be induced by intracortical microstimulation (ICMS) in area 18 of adult cats (3). ICMS-enforced synchronous cortical activity leads to an enlargement of the cortical representational zone at the ICMS-site and an extensive restructuring of the entire OPM layout up to several millimeters away. Here we describe how this plastic reorganization is related to changes in the number and distribution of pinwheels (PW).

OPMs were recorded by means of optical imaging of intrinsic signals in area 18 of adult anaesthetized cats before and after 3 hrs of ICMS. ICMS consisted of charge-balanced current pulse trains (13 pulses at 300 Hz, 6-12 mA, 200 ms duration) delivered at 1 Hz without parallel visual stimulation.

We found that ICMS altered the complete layout and structure of OPMs. We analyzed density and stability of PWs before and after ICMS. Immediately after ICMS, PW density was significantly increased. However, this increase was not due to the fact that some new PWs emerged at new locations. Only a small fraction of the pre-PWs turned out to be stable. Instead, a large number of PWs disappeared and a large number of new PWs emerged, giving rise to dramatic changes of PW locations. This PW mobility continued up to 18 h after termination of ICMS. Accordingly, the recovery of PW locations was incomplete. The finally observed PW pattern consisted of newly emerged PWs and those that came back to their initial locations. These results extend our previous optical imaging results concerning plastic changes of OPMs and substantiate our notion of profound mobility of PW locations even in adult visual cortex.

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556 Learning of natural and synthetic biological motion

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Human body motion presented as point-light stimuli can be readily recognized. Psychophysical experiments show that these impoverished stimuli are sufficient for the discrimination between different actions and even for the extraction of the gender and other details of the moving actor. Additionally, a few studies indicate the capability of humans to learn to discriminate different styles of natural movements (e.g. gaits or sports movements). However, it remains unknown whether this learning is based on innate templates for biological movement patterns, or if humans can learn new representations of arbitrary complex movements. We address this question by investigating whether subjects can learn artificial biological movement stimuli.

Methods: The stimuli used in this study were generated by linear combination of prototypical trajectories in space-time using spatio-temporal morphable models (Giese & Poggio, 1999). We created two different classes of stimuli: (A) Stimuli derived by linear combination of dissimilar natural movements (e.g. walking, kicking and dancing). (B) Stimuli generated by animation of an artificial skeleton model that is highly dissimilar from naturally occurring body structures. The joint angle trajectories of the skeleton were given by linear combinations of synthetic sinusoidal trajectories. Their amplitudes and frequencies were approximately matched with the joint trajectories of human actors during natural movements. For both classes, several stimuli were created which served as prototypes for the morphing procedure.

Subjects had to discriminate within one class between pairs of these stimuli that were defined by linear combinations with slightly dissimilar weights of the prototypes. The trajectories were presented as normal point light walkers (PLW), and as point light walker with position jitter (PLWJ). The PLWJ were generated by adding random displacements of the dots along the skeleton of the walker in each frame (similar to Beintema & Lappe, 2002). Each subject took part in three test blocks that were intersected by two blocks of training. Feedback was provided only during training.

Results: Subjects trained with stimuli derived from natural movements (class A) learned the discrimination between novel patterns very quickly, regardless of the stimulus type (PLW/PLWJ). If the training stimuli were rotated 90 deg in the image plane against the test stimuli, we observed transfer of the learned representation only for the normal PLW but not for the PLWJ stimuli.

The completely artificial stimuli (class B) were only presented as PLWJ. Subjects were able to learn these stimuli equally fast as the natural stimuli (class A). In addition, we found the same view dependence as for the natural stimuli.

Conclusions: (1) New templates for movement recognition can be learned very quickly. (2) Learning affects at least two different levels of representation (local and holistic). (3) The learned holistic representations seem to be view-dependent. (4) There seem to be no significant differences between the learning process for stimuli derived from artificial and natural movements.

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Processing of natural scenes in cat V1 557

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The question how stimulus features are encoded in neuronal responses has been at the heart of neuroscience for many years. In the visual system the representation of stimulus features seems well understood given that firing rates in V1 encode stimulus properties such as orientation, contrast, spatial frequency etc. Concerning the representation of global stimulus properties, two prominent hypotheses have been proposed. One suggests that neurons encoding features of the same object are bound by correlated in- or decreases in firing rate. The other hypothesis suggests that such neurons share temporal response properties such as synchronization. However for both of these there exists conflicting evidence. Furthermore studies addressing this question used artificial stimuli such as bars or gratings whose statistical properties of these differ markedly from those of the natural visual input.

To study the impact of global stimulus properties on synchronization and firing rates we measured local field potentials and multiunit activity in the primary visual cortex of awake cats. As stimuli we use gratings, natural movies and movies with altered statistical properties such as pink noise.

The drifting grating elicits a temporally uniform response which is limited to a restricted range of frequencies; a decrease in activity between 5-20Hz and an increase between 30-80 Hz. For all other stimuli, especially the natural movies, a much larger frequency range is activated (increases between 30 and >300 Hz). Remarkably, the activity pattern for natural movies does not differ significantly form that for the pixel noise. For wavelet-manipulated movies, the relative contribution of different frequencies to the activity is very similar though total response amplitude is decreased. The strongest differences in response strength between stimuli is found in the γ range. In the temporal domain the activity for natural movies is very non-uniform, characterized by a strong transient response followed by very irregular in and decreases. We show that these correlate well with high amplitude motion components in the movies. To disentangle the relation of motion and spatial structure we used stimuli following uniform and such following high frequency jitter motion extracted from natural movies. The effect of simple vs. complex spatial structure is independent of the superimposed motion. However, both motion and spatial structure affect the temporal response properties.

Complementing the local field potentials we measured multi unit spikes in two subjects. Comparing the average firing rates for the different stimuli we find that the natural movies elicit only slightly stronger responses than the pixel noise.

We show that the activity patterns elicited by natural movies are very different from those caused by classical visual stimuli such as gratings. Furthermore we find that the local field potential responses are to a large part determined by the second order statistics of the input only and only weakly reflect the higher order properties. The highly

irregular motion of natural movies strongly shapes the temporal properties of the cortical responses. However neither a rate code nor the temporal response properties uniquely reflect the statistical structure of the stimulus.

558 Does luminance contrast contribute to a saliency map for overt attention?

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In natural environments, humans select a subset of visual stimuli by directing their gaze to locations attended. In previous studies it has been found that at fixation points luminance-contrast is higher than average. This lead to the hypothesis that luminance-contrast makes a major contribution to a saliency map of visual overt attention, consistent with a computation of stimulus saliency in early visual cortical areas. We re-evaluate this hypothesis by using natural and modified natural images to uncover the causal effects of luminance-contrast to human overt visual attention: (1) We confirm that - viewing natural images - contrasts are elevated at fixation points. This, however, only holds for low spatial frequencies and in a limited temporal window after stimulus onset. (2) However, despite this correlation between overt attention and luminance-contrast, moderate modifications of contrast in natural images do not measurably affect the selection of fixation points. Furthermore, strong local reductions of luminance-contrast do not repel but attract fixation. (3) Neither contrast nor contrast modification is correlated to fixation duration. (4) Even the moderate contrast modifications used fall into the physiologically relevant range, and subjects are well able to detect them in a forced choice paradigm.

In summary, no causal contribution of luminance-contrast to a saliency map of human overt attention is detectable. In conjunction with recent results on the relation of contrast sensitivity of neuronal activity to the level in the visual cortical hierarchy, the present study provides evidence that - for natural scenes - saliency is computed not early but late during processing.

559 Functional Segregation of Visual Pathways by Learning from Natural Image Sequences

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Sensory systems extract multiple features in parallel, and meet this requirement with distinct populations of neurons that are selective to one property of the stimulus while being relatively non-selective (invariant) to another property. In this way, several populations can resolve a set of features independently of each other, and thus achieve a parallel mode of processing. This raises the question how an initially homogeneous population of neurons segregates into groups with distinct and complementary response

properties. Using color image sequences recorded from a camera mounted to the head of a freely behaving cat, we train a network of neurons to achieve optimally stable responses, that is, responses that change minimally over time. This objective leads to the development of colour selective neurons. Adding a second objective, de-correlating activity within the network, a subpopulation of neurons develops with achromatic response properties. Colour selective neurons tend to be non-oriented while achromatic neurons are orientation-tuned. The proposed objective thus successfully leads to the segregation of neurons into complementary populations that are either selective for colour or orientation.

Extended DOG model for relay cells in cat lateral geniculate nucleus

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Visual information predominantly reaches the cortex via the lateral geniculate nucleus (LGN) in mammals. Information is transmitted by the relay cells of the LGN, which receive excitatory input from retinal ganglion cells, and project excitatorily to the visual cortex. Relay cells are inhibited by local interneurons and by cells in the thalamic reticular nucleus (TRN). LGN cells further receive, at least in the anatomical sense, massive feedback from cortex. This feedback has been shown to enhance inhibition of relay cells when bipartite bar or drifting-grating stimuli are used[1].

Retinal ganglion cells and LGN relay cells have small, roughly circular receptive fields which commonly have been described mathematically by the difference-of-Gaussians (DOG) model. The DOG-model is an example of a descriptive model. The purpose of *descriptive* modeling is to summarize experimental data compactly in a mathematical form. In *mechanistic* modeling, on the other hand, one attempts to account for nervous system activity on the basis of neuronal morphology, physiology and circuitry.

Recently, we have presented a general linear rate-based mechanistic model for the signal processing in the LGN circuit, taking into account all feedforward (from retina and LGN interneurons) and feedback (from TRN and cortex) connections[2]. Here we consider a reduced version of this circuit model where (i) the effects of the feedforward excitation and inhibition are described by the DOG-model, (ii) the feedback effects from TRN are neglected, and (iii) only the inhibitory "relay cell-cortex-interneuron-relay cell"-loop in the cortical feedback is considered. We then derive a model for the spatial receptive field taking into account cortical feedback from an entire set of orientation-selective cortical cells covering all orientation angles. This makes the overall cortical feedback circularly symmetric.

In (the Fourier transformed) *k*-space the new model, which we call the *extended* DOG-model (eDOG), is given by a fraction, the numerator of which corresponds to the standard DOG-expression, while the denominator accounts for the extra inhibition from the cortical feedback loop. The eDOG receptive-field in position-space can be found by a inverse Fourier-transform which, due to the circular symmetry of eDOG, in the general

case is given by a one-dimensional integral. For moderate feedback strengths the inverse Fourier transform can be performed analytically. The receptive-field function is then found to be given by an infinite series of DOGs, with the first term corresponding to the feedforward DOG. In the series each consequtive term has larger spatial widths and smaller weights than the previous. The number of series required to achieve convergence depends on the cortical feedback strength; the smaller the strength, the fewer terms are needed.

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561 Effect of CYP26 over-expression on development of the retinotectal projection of the chick

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In embryonic development of the vertebrate eye gradients of transcription factors determine the two orthogonal topographic axes, dorsal-ventral and temporal-nasal. These factors are believed to form retinal coordinates as the underlying basis for the topographically organized visual projection. We test the hypothesis that a graded distribution of retinoic acid (RA) is instructive for the establishment of a dorsal-ventral polarity. The consequence of changing RA levels on regional patterning within the retina has not been clarified. To study the effects of RA as a retinal determinant we manipulated its distribution in E2 chick embryos with (1) ectopic expression of the RA-inactivating enzyme CYP26, (2) injections of RA and (3) pharmacological inhibition of RA synthesis.

Expression vectors containing the genes of the cytochrome P450-oxidase CYP26 and GFP were introduced into the prosencephalic neural epithelium of stage 10 embryos by electroporation (20 electric pulses of 10V DC, 50 ms duration each, current below 20 mA). Other embryos received injections of 100 nM RA each or 100 nM citral. Embryos were then cultivated 24 hours in ovo. Successful introduction and subsequent expression of the vectors was verified by visualizing the GFP-label at embryonic stage 18. Thereafter, the embryos were fixed and sectioned on a cryostat microtome. Immunohistochemistry for the transcription factor Pax2 was performed on sections and wholemounts following standard immunohistochemical procedures. Intensity and the distribution of the Pax2 label, which originated at the ventral pole of the eye and extended dorsally, were measured. In addition, the expression of the cytokine BMP-4 was assessed with quantitative RT-PCR and *in situ* hybridization.

The results show that neither CYP-26-electroporation nor RA-treatment produced a significant shift in Pax2 expression. The intensity of the Pax2-label was also not significantly altered compared to the controls. Preliminary data from *in situ* hybridization and RT-PCR, however, indicate that RA downregulates BMP-4, suggesting that RA acts as a ventralizing factor upstream of BMP-4 but not of Pax-2.

Lateral competition: The interplay of inhibition and excitation in primary visual cortex on the development of topographic projections and ocular dominance maps 562

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The development of visual cortex feature maps, e.g. ocular dominance maps, topographic maps and orientation maps, have been extensively investigated and numerous modeling studies have examined their development. Two prevalent classes of Hebbian models are correlation based learning models (CBL) and self-organizing maps (SOM), which have both been proposed to explain the activity driven formation of cortical maps. Both models differ significantly in the way lateral cortical interactions are treated leading to different predictions for the formation of receptive fields. In previous studies (Piepenbrock & Obermayer, 2000, Wiesing & Obermayer, 2001) we investigated a class of models which are characterized by a variable degree of lateral competition and which have the CBL and SOM models as limit cases. We showed that there exists a critical value for intracortical competition below which the model exhibits correlation based learning properties and above which feature mapping sets in. This mechanism of variable degree of competition, formulated through a softmax-function, allows analytical predictions, but due to his mathematical formalization do not find its direct equivalent in biology. Using a firingrate-based mean field model (Bartsch et al. 2000) we examine the development of afferent synaptic weights between two input layers (left/right LGN ON cells) and one cortical layer of neurons (V1 input layer, one excitatory and one inhibitory cell population) in a more biological realistic way. We motivate each used parameter in detail and show in which parameter regimes localized receptive fields and ocular dominance bands will form.

Development of thalamocortical visual circuits: A model based on the neurotrophic hypothesis 563

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Neurons in primary visual cortex of higher mammals possess typical response properties such as ocular dominance and orientation preference. Pioneering physiological and anatomical studies have shown that activity plays a major role in shaping these response properties. Recently it has been shown that neurotrophins act as molecular signals that translate activity into structural and functional changes of neural connections. Motivated by this experimental finding and to study the role of neurotrophin in plasticity, we present a model for the development of thalamocortical visual ciruits based on the hypothesis that neurotrophins are released in an activity dependent manner at each synapse and that diffusion of these released neurotrophins through both intracellular and extracellular space,leads to correlated changes in different neurons. We assume a two layer structure,

the output layer consisting of a two dimensional array of NxN cortical neurons receiving input from an input layer which consists of two 2D arrays of MxM LGN neurons. The two arrays in the input layer corresponds to either ON/OFF or left eye/right eye LGN neurons. Initially at each location there are two types of synapses because of two types of input populations. The arborization of a particular cell is limited in space and is controlled by an arbor function. The growth or decay of synaptic connection depends upon the uptake of neurotrophic material. The weight update equation consists of three terms: (i) a term describing intrinsic uptake of neurotrophin (some portion of it does not diffuse or does not take part in competition), (ii) a second term describing the crosstalk between nearby synapses, (iii) a third term which describes competition between different types of synapses at each location because of limited resources. We find that with this form of model equations, typically if one cell population withdraws from a postsynaptic site the competing population progressively takes over. We also find that out of the total amount of neurotrophin released, only some portion should be allowed to diffuse. If all the released material, is allowed to diffuse (i.e if there is only no.(ii) term in weight update equation) then there is no growth of synaptic strengths. It was also observed that depending upon the correlation between different input populations for proper segregation to take place, the portion of substance which should be allowed to diffuse also changes. In case of similar correlated activities the amount needed is less in comparison to the case when the input population have anti-correlated activities. We find that inputs segregate starting from the homogenous state, and we obtain ocular dominance bands, if we considered input population to be left and right eye type, and segregated ON and OFF regions in simple cell receptive field, if we considered inputs from ONtype and OFFtype of LGN neurons.

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564 Regulation of interneuronal voltage-gated potassium channels Kv3. 1b and Kv3. 2 expression in rat visual cortex

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The GABAergic interneurons in the rat neocortex represent a heterogenous population concerning morphological, neurochemical or physiological phenotype. The parvalbuminergic basket and chandelier cells for instance inhibit pyramidal neurons by axosomatic and axoaxonic synapses, resp., and have a so called “fast-spiking” (FS) electrophysiological phenotype. It is based on the expression of two voltage-gated potassium channels, termed Kv3.1b and Kv3.2. The heterogenous channel formation allows a fast repolarisation of the membrane, which allows the neurons to fire groups of non-adapting action potentials at high frequencies. FS-interneurons are pacemakers for the pyramidal neurons allowing excitatory activity and information processing only in the pauses between inhibitory bursts. The electrophysiological properties of FS neurons in the model system of organotypic cultures (OTC) have been previously characterized (Klostermann and Wahle, 1999). We know from our other studies that environmental factors like neuronal activity, afferent innervation or neurotrophic factors influence the development and molecular differentiation of interneurons (Wahle et al., 2000; Wirth et al., 1998). For

this reason we investigate here whether the expression of the Kv3 channels depends on environmental factors. The expression of the Kv3.1b/3.2 mRNAs and proteins are quantitatively analyzed in postnatally developing visual cortex *in vivo* and in OTC using PCR, Western Blot and *in situ* hybridization to characterize the cell types *in vitro*. Kv3 mRNA and protein increase with age *in vivo* and in OTC. However, developmental upregulation of expression in OTC is faster and reaches higher levels than *in vivo*. Kv3.1b mRNA expression does not depend on neuronal activity, but Kv3.2 mRNA expression does since expression is severely reduced in activity-deprived OTC. In spontaneously active OTC, leukemia inhibitory factor (LIF) and NT4 both accelerate Kv3.1b mRNA expression during the first ten days in culture, however, the factors failed to alter the peak levels present at 20 DIV. In activity-deprived OTC, only LIF increases Kv3.1b mRNA expression. In spontaneously active OTC, both factors fail to influence Kv3.2 mRNA expression during the first 10 DIV, but a responsiveness to NT4 develops with age, and NT4 stimulates Kv3.2 transcription in 20 DIV cultures. Also in activity-deprived OTC does NT4 promote Kv3.2 mRNA in 20 DIV OTC. Further, it is known that aminergic cortical afferents influence interneuronal phenotypes. For instance, dopamine upregulates Parvalbumin expression (Porter et al., 1999). We therefore tested dopamine and serotonin and found that dopamine acutely increases Kv3.1 mRNA, but not Kv3.2 mRNA. In contrast, serotonin fails to alter Kv3.1b mRNA, but reduces Kv.3.2 mRNA. These results reveal that the neuronal activity, trophic factors and aminergic transmitters influence Kv3 mRNA expression, and thus strongly regulate the development of the functional state of visual cortical interneurons.

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Serotonin regulates GAD-65/67 mRNA and protein expression in developing rat visual cortex 565

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We have recently characterized the role of neuronal activity, neurotrophins and afferent systems for GAD-65/67 mRNA and protein expression in visual cortex in organotypic cultures (OTC) and *in vivo* (Patz et al., submitted). The results suggested neuronal activity as major regulator which differentially affects transcription and translation of the two isoforms. However, activity and neurotrophin signalling in OTC failed to bring forth an “organotypic” adult pattern of GAD-65 mRNA and GAD-67 protein expression. This is likely due to the lower network activity in OTC and/or the lack of neuromodulatory afferent input.

One important afferent system arises from serotonergic midbrain neurons and selectively targets neuropeptidergic and calbindin-containing interneurons, and serotonin receptors are expressed in interneuron types and pyramidal cells. Serotonin mediates excitation of interneurons (Kawa, 1994; Rörig et al., 1997) thus enhancing GABAergic

transmission which is excitatory during the early postnatal period (Cherubini et al., 1991). We therefore tested the role of serotonin with a pharmacological approach.

PCR revealed that 5-9 DIV Serotonin treatment failed to alter the levels of both GAD mRNA´s, but it increased the GAD-67 protein expression to levels observed in age-matched cortex *in vivo* without causing marked changes of the GAD-65 protein. Since serotonin increases the network activity, it argues for a use-dependent translation of GAD-67 protein.

Moreover, early serotonin influence had a priming function for GAD-65 mRNA expression. Untreated 'adult' OTC normally express 2-fold higher levels of GAD-65 mRNA than adult cortex *in vivo*. A temporally limited (5-9 DIV) exposure to serotonin caused a selective reduction of the level of GAD-65 mRNA to about *in vivo* levels at 20 DIV. The GAD-67 mRNA level remained unchanged and protein expression of both isoforms was unchanged compared to control OTC. These results clearly showed that a higher network activity as evoked by serotonin signalling during a period of molecular plasticity selectively influences the developmental profile of the GAD-65 transcription. In contrast, higher network activity must likely be continuously present to stabilize GAD-67 protein at 'organotypic' levels.

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566 $GABA_C$ Receptors: Developmental Regulation of Expression and Electrophysiological Profiles in Organotypic Cultures of the Superior Colliculus

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The superior colliculus (SC) is one of the brain areas besides the retina, where $GABA_C$ receptors are abundant in particular in the superficial gray layer (Wegelius et al. 1998. Eur. J. Neurosci. 10, 350-357, Boue-Grabot et al. 1998 J Neurochem. 70(3), 899-907). The regulation of postnatal developmental expression of the σ-subunits (σ1-3) in the SC and other CNS areas is unknown, and the expression may well be cell-autonomous. However, since the SC receives retinal and visual cortical input amongst others, an obvious possibility is a regulation of expression by environmental factors like light, afferent input or neurotrophins. Further, the functional role of the $GABA_C$ receptor in the SC is not completely understood. In particular, the source of the GABAergic projection targeting the $GABA_C$ receptors is still unknown.

We set out to clarify these issues using *in vivo* and *in vitro* approaches using molecular, histochemical, and electrophysiological techniques.

First, we analyzed the developmental time course of σ-subunit mRNA expression with PCR in SC, visual cortex, and hippocampus *in vivo*. We found that σ1 and σ2 expression increased postnatally in all three structures to reach adult levels between P30-P60. Rho 3 mRNA was present in retina, but almost absent in central structures. Second, we compared σ1 and σ2 expression in SC *in vivo* and in organotypic monocultures (OTC) deprived of afferent input. We found that σ1 and σ2 expression in OTCs increased to levels higher than *in vivo*, which might suggest the existence of negative modulatory influences *in vivo* that are not conserved in OTCs. Third, we analyzed σ1 and σ2 expression in SC, visual cortex and retina of normal and dark reared rats. We found basically the same expression levels suggesting that sensory experience has no role for regulating σ receptor expression.

The PCR studies revealed the expression of σ mRNAs in OTC; however, the question arose whether functional receptor proteins are expressed. Moreover, the OTC model might gain more insight into the functional role of $GABA_C$ receptors in the SC and the question of the GABAergic innervations targeting $GABA_C$ receptors.

For this, we used whole patch-clamp recordings cell in OTCs of the SC. To this end, we concentrated on the characterization of the physiological profile of the $GABA_C$ receptors in the organotypic cultures. We could indeed evoke IPSCs by use of photo-uncaging of GABA. These IPSCs were reduced after application of bicuculline, but abolished completely only with picrotoxin. This suggested the presence of functional $GABA_C$ receptors in these SC cultures.

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Accelerated dendritic development of rat cortical pyramidal cells and interneurons after biolistic transfection with BDNF and NT-4/5 **567**

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Neurotrophins are candidate molecules for regulating dendritogenesis (McAllister 2000). We report here on dendritic growth of rat visual cortex pyramidal cells and interneurons overexpressing BDNF and NT-4/5. Neurons in organotypic cultures were biolistically transfected with plasmids encoding either enhanced green fluorescent protein EGFP, or BDNF/EGFP, or NT-4/5/EGFP either at the day of birth with analysis at 5 DIV, or at 5 DIV with analysis at 10 DIV. Further, pyramidal neurons from spontaneously active or activity-deprived organotypic cultures were biocytin-filled and analyzed later than 30 DIV (adult).

In pyramidal neurons, both trkB ligands increased dendritic length and number of segments without affecting maximum branch order and number of primary dendrites. There was no evidence for exuberant numbers of short perisomatic dendrites selectively after transfection with BDNF. The effectiveness of the trkB ligands depended primarily on

developmental stage. In the early time window only infragranular neurons were responsive. They reach a state of dendritic maturity similar to the 10 DIV control neurons.

During the later time window, neurons in layers II/III became responsive to NT-4/5, but not BDNF. BDNF and NT-4/5 transfectants at 10 DIV had still significantly shorter dendrites than adult pyramidal neurons suggesting a massive growth spurt after 10 DIV. However, segment numbers already were in the range of adult neurons. Although this suggested a role for BDNF, long term activity-deprived and thus BDNF-deprived pyramidal cells developed a dendritic complexity not different from neurons in active cultures except for higher spine densities on neurons of layers II/III and VI.

Transfected multipolar interneurons became identifiable during the second time window. Both trkB ligands significantly increased the number of segments and the branch order towards the adult state when compared to multipolar interneurons obtained by biocytin injections in 22-142 DIV OTC (Klostermann and Wahle, 1999). The dendritic length of interneurons was only marginally increased. The results suggest that early in development BDNF and NT-4/5 can accelerate dendritogenesis in an autocrine fashion. In particular, branch formation was advanced towards the adult pattern in both, pyramidal cells and interneurons.

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568 Diurnal regulation of NT4, LIF and BDNF: Role of sensory experience

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Neuronal activity and sensory experience are known to promote the mRNA expression of BDNF. For instance, BDNF increases in postnatal visual cortex with the increase of visually evoked activity after eye opening (Schoups et al., 1995), and dark-rearing rats from birth prevented this increase, and BDNF mRNA expression also remains low in visual cortex organotypic cultures deprived of spontaneous neuronal activity (Gorba et al, 1999). Further, BDNF is subject to diurnal regulation (Pollock et al., 2001). It is unknown whether the expression of NT4, the second trkB ligand, is regulated by activity and sensory experience, although its expression is not decreasing in activity-deprived OTC. Further, the regulation of expression of the cytokine leukemia inhibitory factor, LIF, has not been studied in these conditions. One possibility to alter visual cortical activity *in vivo* is constant-darkness (CD) which like dark-rearing (DR) is believed to result in lower activity by eliminating phasic visual transients. The opposite treatment is a constant-light condition (CL); it might evoke higher levels of activity. A final possibility is to analyze the expression during the diurnal dark-light cycle (DL; ‘night/day’). We therefore analyzed with PCR the mRNA expression of NT4 and LIF together with BDNF in visual cortex of P40 rats exposed to DL, or CL and CD (for 5 days). Other animals were dark-reared until P45 (DR), and some were reexposed to light for 1 and 4 hours (recovery).

BDNF increased in visual cortex in DL with light ON and declines after light OFF as expected and as reported earlier (Pollock et al., 2001), and this increase did not occur in CL and CD. Hippocampus did not show any modulation of BDNF. NT4 in visual cortex was constant in DL and CD, but was higher during the 'day' in CL. Hippocampus revealed an afternoon peak in NT4 mRNA in DL, but no modulation in CL and CD. LIF in visual cortex remained at the same level during the diurnal period in DL, CL and CD, and was not modulated in hippocampus.

A direct comparison of BDNF mRNA levels at 12:00 in DL, CL, CD and DR revealed that CD levels were reduced by 50% as they were in DR. In recovery, light exposure for 1 h transiently evoked a 3-fold increase. Activity levels thus determine the BDNF mRNA levels. In contrast, NT4 was constant in DL, slightly elevated in CD, and light during the 'night-phase' in CL evoked a downregulation. NT4 was slightly elevated in DR, but declined after 1 and 4 h reexposure to light. LIF in CL, CD and DR was slightly higher than in DL, and transiently increased after reexposure to light.

The results suggest that visual activity influenced the expression of NT4 and LIF although to a lesser extent than BDNF.

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CNTF exerts opposite effects on the expression of opsins in different subtypes of photoreceptors in reaggregated spheres of the chicken retina. 569

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Although several studies have shown an effect of CNTF (ciliary neurotrophic factor) on retinal photoreceptors, it is not clear whether CNTF acts on certain photoreceptors or on all photoreceptor subtypes. For analysing the effect of CNTF on different types of photoreceptor we used histotypic three-dimensional retinal spheres, so-called rosetted spheroids. The opsin expression were investigated by using semi-quantitative RT-PCR in combination with specific primers for opsins of either rods or for red-, green-, blue- and violet-sensitive photoreceptors. Our results demonstrate that CNTF decreases the opsin mRNA expression of red-sensitive cones after 8 days in culture, whereas the number of rhodopsin and green-sensitive opsin transcripts is upregulated. An explanation for this opposite effect of CNTF is probably based on the phylogenetic evolution of opsins. Phylogenetic analysis of opsins has revealed that the similarity between rod and green cones is higher than between red and green cones, respectively. On the assumption that the expression of opsins is correlated with the number of photoreceptors, we postulate that CNTF differentially regulates the development of rods and green cones as compared with red cones. Interestingly, transcripts of blue and violet opsins were not detectable neither in CNTF nor in non-treated cultures. Furthermore, we found that CNTF increases the expression of bcl2 transcripts at later stages of rosetted spheroid development, indicating a possible anti-apoptotic effect of CNTF at least on rods and green cones.

570 Expression pattern of GFRα-4 during development of the chicken retina

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The GDNF family receptors α 1-4 (GFRα 1-4) are prominently expressed during embryonic development in a series of neuronal and nonneuronal tissues. Recently, it has been shown that GFRα-1 and α-2 are also expressed during embryogenesis of the chikken retina. To investigate the possible involvement of GFRα-4 in retinal development, we analysed the expression pattern of GFRα-4 transcripts during the development of the chicken retina. In contrast to other members of the GDNF family receptors, we found that GFRα-4 is temporally co-expressed with the signal transducing component Ret receptor tyrosine kinase. The temporal and spatial expression of GFRα-4 is developmentally regulated in retinogenesis and is first detected in cells of the ganglion cell layer at E6. As development of the retina precedes, the expression of GFRα-4 extends to cells of the inner half of the inner nuclear layer and to horizontal cells. Later on, GFRα-4 expression is found in additional cells of the outer half of the inner nuclear layer as well as in a subpopulation of photoreceptors of the ONL. Also, the onset of GFRα-4 expression is first detectable in the central portion of the retina, whereas GFRα-4 expression in the peripheral retina is temporally delayed by two days, reflecting the central-to-peripheral gradient of differentiation during retinal development. These data suggest that GFRα-4 plays an important role during the formation of the chicken retina.

571 Complete postnatal degeneration of photoreceptors as a consequence of distorted IPL formation in an AChE knockout mouse

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The major cell types and retinal layers of the vertebrate retina are formed in defined spatio-temporal sequences. Cells of the inner retina differentiate first; dendrites of amacrine and ganglion cells synapse with processes of bipolar cells in a restricted neuropil area called inner plexiform layer (IPL). The IPL is a highly organised spatial network subdivided by multi-fold horizontal neuropil sublaminae. Their development is not understood; however, an easily traceable early hallmark is the establishment of two acetylcholinesterase (AChE)-positive sublaminae, suggesting that AChE has a network-organising role in the retina (Layer et al., 1997). Acetylcholine together with the cholinergic system is decisive to establish spontaneous electrical activity in the retina. We used an AChE knockout mouse, which can survive until adulthood (Li et al., 2000; Xie

et al., 2000; Duysen et al., 2002), to study the role of AChE in retinal network formation. In the AChE KO mouse, no AChE-positive sublaminae are detectable. Comparing the KO and wild-type mouse, we have followed sublaminae formation by immunostaining of two major calcium binding proteins, calretinin (CR) and parvalbumin (PA). A highly regular arrangement of three major CR+ sublaminae established during the first 15 postnatal days is entirely disturbed in the KO mouse. Detectable already from P1 onwards, processes of amacrine and ganglion cells diffusely criss-cross throughout the IPL. In contrast, PA+ cells present a highly irregular, non-laminar IPL pattern in the wild-type, but in the KO mouse their processes become regularly arranged within two "novel" sublaminae. In the outer retina of the AChE KO mouse from P1 to approximately P20, photoreceptors are arranged regularly and at a normal rate. During the following 40 days, however, the OS completely deteriorated. With a delay, the photoreceptor somata also disappear due to apoptosis, as established by TUNEL staining. At P28, the first apoptotic nuclei in the ONL are discernible, rapidly increasing to its maximum by P61, and then decline. By P86, only few TUNEL+ cells remain, while the photoreceptor layer is non-existent anymore. Only after complete disappearance of the ONL, cells in the inner retina increasingly become apoptotic. Therefore, photoreceptor degeneration follows only after distortions in the IPL have become evident. The phenomenon described here resembles several retinal degeneration models, e.g. the rd-mouse, rcs rat, or models of Usher syndrome, however, in contrast to these models, here PR degeneration is a consequence of earlier changes in the inner retina (cf. also Banin et al., 1999). Furthermore, these findings i) strongly support a major role of AChE in cell process formation, pathfinding and synaptogenesis in the inner retina. AChE ii) affects very early processes of retinal network formation, which iii) ultimately can lead to photoreceptor degeneration. iv) The related enzyme butyrylcholinesterase (BChE), albeit increased in the KO mouse, cannot – as generally supposed - replace AChE in these functions.

Glial cell line-derived neurotrophic factor promotes differentiation and survival of rod photoreceptors in reaggregated spheres of the chicken retina

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To investigate the role of glial cell line-derived neurotrophic factor (GDNF) on the development of photoreceptors, we used reaggregated histotypic spheres of the chicken E6 retina. Under rotation conditions, so-called rosetted spheroids were formed by aggregation of dissociated retinal cells, followed by their proliferation, migration and differentiation. Rosetted spheroids were cultured for 10 days under serum-reduced conditions either in the absence or presence of 50 ng/ml GDNF. At early stages of culture, GDNF significantly increases and sustains the rate of proliferation. Possibly caused by this proliferative effect, we observed an increase in the number of rod photoreceptors, whereas cone photoreceptors were not affected. Moreover, in GDNF-treated cultures, rod photoreceptors differentiate earlier than in non-treated cultures. When spheroids were raised under serum-reduced conditions in the absence of GDNF, rod but not cone photo-

receptors undergo apoptosis. By supplementing GDNF, apoptosis of rod photoreceptors was decreased (31% to 6% at 8 days in culture, 71% to 3% at 10 days in culture). Our data indicate that GDNF regulates the proliferation, differentiation and survival of rod photoreceptors.

573 Organisation of the Visual Cortex in Human Albinism

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Purpose: The albino visual cortex receives abnormal input from the ipsilateral visual field (Guillery, 1986). It therefore serves as an ideal model to study principles that under-lie self-organisation and re-organisation of the visual cortex. To investigate how the abnormal input from the ipsilateral visual field is topographically mapped in the visual cortex of human albinos we applied retino-topic mapping fMRI procedures (Engel et al., 1997).

Methods: Six subjects (2 normal controls and 4 subjects with albinism) underwent T2* weighted MRI scanning of the occipital lobe during visual stimulation (checkerboard reversal at 6 Hz; visual field size: 22° radius; expanding rings and rotating wedges for eccentricity and polar angle mapping, respectively). A volume comprising twelve 128 x 128 voxel images (voxel size: $1.8 \times 1.8 \times 4$ mm; plane orientation perpendicular to calcarine sulcus) was acquired every 3 s, a total of 72 such volumes was collected for each experiment. After Fourier- and correlation analysis of each voxel's time-series, fMRI data were projected to the flattened representation of T1 weighted images of the occipital lobe.

Results: We report four main findings in the subjects with albinism: (1) We found an abnormal cortical representation of the ipsilateral visual field. (2) This abnormal representation is evident in the central visual field (central 7-15°) and absent in the periphery. (3) Normal and abnormal representations were identified within similar boundaries of the early visual areas. (4) The abnormal cortical repre-sentation of the ipsilateral visual field is mirror-symmetrically superimposed onto the normal representation of the contralateral visual field in both striate and extra-striate cortex.

Conclusions: The majority of animal studies on albinism report that the mis-match of visual information at the cortical level is resolved either by re-ordering the geniculostriate projection or by suppressing the abnormal cortical input. Our results suggest that different mechanisms to resolve the sensory conflict are adopted in human albinism. We propose that modifications of the cortical circuitry that normally integrates binocular input must, in subjects with albinism, be modified or fail to develop.

Shape and spacing of orientation columns in ferret visual cortex 574

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Because orientation selective responses in ferret visual cortex develop several weeks after birth, ferrets have become an important model animal for studying the role of experience and activity in the development of visual cortical circuitry[1,2]. To enable a detailed comparison of orientation maps (OMs) emerging in ferrets with differing visual experience, we adapted a previously developed method for the quantitative characterization of OMs[3], and applied it to the analysis of OMs obtained by intrinsic signal imaging in ferrets (N=83 hemispheres). The method is based on the wavelet representation of difference images of the patterns of cardinal (0°,90°) and oblique (45°,135°) iso-orientation domains (IODs). Using this representation we calculated parameters measuring the average spacing of IODs and the tendency of IODs to form elongated bands (bandedness). Both spacing and bandedness of IODs varied considerably in different animals. The spacing ranged between 0.8mm and 1.1mm, and the bandedness between 0.3 and 0.45, differing by more than 50% among individuals. Spacing and bandedness were only weakly correlated. The comparison of results obtained from single condition and difference images indicated that spacing and bandedness can be estimated with a relative precision of about 5% and 10%,

respectively. In addition, we observed that cardinal and oblique IODs differed substantially in their size and bandedness (up to 20%). We conclude that wavelet analysis successfully captures the main inter-individual differences between OMs in different ferrets. Supported by the Volkswagen Stiftung and the Max-Planck-Gesellschaft.

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[2] Coppola et al. 1998, PNAS, 95.
[3] Kaschube et al. 2002, J.Neurosci. 22.

Developmental plasticity in spectral sensitivity and processing in the cichlid fish *Aequidens pulcher* 575

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INTRODUCTION

Vertebrate color vision has long been regarded as determined almost exclusively by genetic factors. Experimental manipulations of the visual environment had induced no or little effects on spectral processing and/or color vision in a variety of vertebrates ranging from fishes to primates. In a series of studies using stringent rearing and testing

conditions, we have observed developmental changes in the color vision system of the Central American cichlid fish *Aequidens pulcher* (blue acara). Rearing fish under spectral deprivation and different intensities of white light produced effects on all levels of spectral processing studied, which ranged from sub-cellular morphology to behavior. Here we present a summary of the results.

MATERIAL AND METHODS

Fish were reared under spectrally narrow-banded ("monochromatic") lights from early larval stages until, as adults, they were used in the experiments. Control groups were reared under different levels of white light. Structures and processes relevant to spectral sensitivity and processing were studied with light, confocal, and electron microscopy, microspectrophotometry, immunohistochemistry, intracellular recordings and dye injections, as well as analyses of a kind of innate behavior, the optomotor response.

RESULTS

Rearing blue acara under monochromatic light of short wavelength (blue or violet light) induced a number of effects: (i) reduction of morphological changes in cone photoreceptor synapses between light and dark adapted states, (ii) elongation of the outer segments of long- and middle-wave-sensitive cones, (iii) selective elimination of short-wave-sensitive cones, (iv) modification of horizontal cell spectral responses, and (v) changes in the efficiencies of different spectral ranges of light to drive the optomotor response. We also found the following differences between animals reared under bright and dim white lights: (i) cone – horizontal cell connectivities, (ii) horizontal cell spectral responses, and (iii) the optomotor response.

DISCUSSION

Our results show that spectral processing in *A. pulcher* is subject to developmental plasticity. Some of the induced effects (e.g. elimination of short-wave-sensitive cones in light of short wavelength) seem to be brought about by compensatory mechanisms which aim at balancing the signals from short- vs middle- and long-wave-sensitive cones. The effects observed on higher levels (e.g. increased efficiency of blue light to drive the optomotor response after rearing in short-wave light) indicate that the mechanisms are more complex than a simple short vs long wavelength compensation of spectral sensitivity. It is therefore likely that several levels of spectral processing are involved in the developmental fine-tuning of the visual system of the blue acara.

Rearing in dim white light may have induced spectrally selective effects because long-wave-sensitive cones are more affected by thermal noise than middle- and short-wave-sensitive cones.

The difference between previous reports and our results may be due to the fol-lowing key features: (i) we reared fish under controlled conditions for long times (usually at least a year), (ii) the fish were not exposed to spectral environments other than the rearing lights prior to the experiments, and (iii) we used an innate behavior that did not require training.

Distortions in Retino-cortical Magnification Factor caused by Cortical Folding

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One of the fundamental organizing principles of the cortex is its topographic mapping of sensory input. It often occurs that sensory inputs are mapped onto the cortical surface such that neighboring neurons code similar features and therewith building feature categories. The simplest example is that spatial neighborhood in the retina is preserved in many visual cortical areas, leading to so-called retinotopic maps. In this case the mapped feature is simply the spatial location of the visual input signal. Other examples are tonotopic organization in primary auditory cortex, or--a more abstract one--the lay-out of orientation selective cells in primary visual cortex (V1).

Retinotopic mapping still allows almost arbitrary metrical distortions and indeed one knows since long that there is a large functional over-representation of the fovea in V1. It was argued that this merely reflects the density of retinal ganglion cells but also that there is an additional magnification in the retino-cortical pathway. Independent of this debate, there is a neglected local effect on the retino-cortical magnification factor resulting from the fact that the cortical mantle is a highly convoluted surface. Variations in Gaussian surface curvature imply inevitable metrical distortions in the retino-cortical magnification and this must influence visual data processing in some way. How self-organized maps develop in the presence of such distortions may lead to mechanistic conclusions.

Local saddle-shaped, i.e., hyperbolic surfaces are characterized by a neighborhood around a point that increases exponentially with its radius, while flat (Euclidean) spaces obey a much slower power law. Implying a

constant cortical column density, a much larger number of cortical columns assemble locally around saddle-shaped cortical folds compared to flat cortical areas. The most prominent example of a hyperbolic cortical patch in the primary visual cortex is at the entrance of the sulcus calcarine on the medial side of the occitipal lobe. The representation of the fovea is close to the occipital pole and parafoveal and peripheral V1 extents towards the medial surface, mainly lying in the calcarine sulcus but also approximately one-third of it onto the surface of the cuneus and lingual gyrus.

There is first evidence coming from distortions of typical forms of transient traveling scotoma for a measurable curvature effect on retino-cortical mapping. The cortical magnification factor along the horizontal meridian in parafoveal V1 seems to be increased compared to meridians in lower and upper visual hemifield. Self-Organizing maps on non-Euclidean spaces are yet little explored and new research may be inspired by the presented findings.

577 Retinal lesion induced plasticity in mouse visual cortex

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The aim of this work is to establish a functional assay of plasticity in mouse visual cortex based on retinal lesions. Although the established models of *in vivo* cortical plasticity are firmly rooted in studies of cat and monkey visual cortex, these species lack the possibility of genetic intervention. Here we report on the development of a murine model for chronic study of cortical changes following localised removal of retinal input, by means of optical imaging of intrinsic signals, electrophysiology and anatomical techniques. Combining this model with genetic approaches allows us to assess the role of candidate genes in this form of plasticity.

In other species, restricted removal of retinal input results in a large reorganisation of functional topography within the deprived region of the visual cortex, which is accompanied by physiological, molecular and anatomical changes. Following a retinal lesion, deprived neurons become responsive to novel positions in visual space during subsequent weeks and months. In cat visual cortex this functional reorganisation is accompanied by terminal sprouting of horizontal connections in the upper layers (Darian-Smith and Gilbert, 1994). However, the exact time course and the cellular mechanisms underlying this functional reorganisation of retinotopy remain largely unknown.

Retinal lesions were carried out in ketamine/xylazine anaesthetised mice by means of laser induced photo-coagulation under visual guidance through an adapted operating microscope. Lesions were typically circular (~25 degrees in diameter) and localised to the upper retina, one papilla-diameter away from the papilla. The consequences of retinal lesioning were assessed chronically in the primary visual cortex over several optical imaging sessions, starting a few days after lesioning and spanning a period of several months. Square-shaped (~20 degrees) gratings were used for mapping of cortical retinotopy through the skull of halothane anaesthetised mice.

In non-lesioned animals, neighbouring stimuli in visual space evoked neighbouring activity patches of similar size, which when combined yield a continuous map of functional retinotopy outlining the extent of the primary visual cortex. Within three days post lesioning, stimuli presented to the lesioned part of the retina evoked little or no activity, producing a clear cortical scotoma in maps of retinotopy, and indicating that the cortical areas representing the lesioned retinal positions were initially functionally silent. Partial filling in of the functional scotoma was seen by four weeks post lesioning, showing that some reorganisation had already occurred. A smaller region of weaker activity was still detectable up to three months after lesioning, implying that reorganisation in the cortical representation of the lesion was still incomplete.

We are currently investigating whether the reorganisation observed with intrinsic imaging can be corroborated by single unit recordings, and whether it is reflected in changes of horizontal connectivity in the upper layers of the cortex.

Optical Imaging Reveals Retinotopic Map Changes in the Visual Cortex of Ephrin-A Deficient Mice

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Ephrins and their Eph receptors are important guidance cues in the developing visual system. Using functional mapping by optical imaging we have previously shown that Ephrin-A5 knockout mice show slight distortions of the retinotopic map in the primary visual cortex. Since it is known that other members of the Ephrin-A class are also involved in mapping in the visual system, we went on to analyze retinotopic maps in the visual cortex of mice with a functional deficiency for all A-Ephrins. In this mouse mutant a fusion protein consisting of the EphA5 extracellular domain and the Fc portion of human IgG1 is expressed from the tau-locus. The antibody-like fusion protein binds and inactivates all A-Ephrins simultaneously, thereby overcoming a potential functional redundancy.

We used optical imaging of intrinsic signals to map the cortical response to grating stimuli presented at 21 adjacent but non-overlapping positions in the visual field. Each stimulus activated a corresponding region in area 17, thus allowing us to reconstruct the complete retinotopic map in this area. Since the retinotopic maps were highly reproducible between mice, we were able to average maps from several animals, which greatly facilitated the quantitative comparison between different groups of mice.

Overall, adult Ephrin-A deficient mice had a grossly normal retinotopic map, but the map was clearly compressed in cortical regions containing the representation of the peripheral visual field. Somewhat surprisingly, the mapping abnormalities in this mouse were not much stronger than in the Ephrin-A5 knock-out mouse. Thus, other guidance mechanisms must be able to compensate largely for the defect in the Ephrin-A system. Importantly however, experiments in very young mice (around P17) seem to indicate that during early development the retinotopic maps in Ephrin-A deficient mice exhibit much stronger abnormalities: the overall shape of the map is different, and the sizes of cortical regions activated by individual stimuli are always larger than in wildtype animals, suggesting less accurate mapping in the cortex. These results indicate that initial mapping errors caused by the absence of axonal guidance cues might be corrected later in development, possibly by activity-dependent mechanisms. We are currently performing tracing experiments to test whether the observed changes are caused by projection errors on the level of the lateral geniculate nucleus, the visual cortex, or both.

579 Long-term *in vivo* 2-photon microscopy of morphological changes in mouse visual cortex induced by retinal lesions

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Although much is known about functional plasticity in the postnatal cerebral cortex, many questions remain about the underlying structural correlates. These include the degree of morphological stability of individual neurons and synapses during and after development, and the characteristics of morphological changes in response to sensory deprivation. Such structure-function relationships can only be properly characterized by observation over multiple timepoints in the living brain.

To this end, we developed an *in vivo* 2-photon microscopy approach for long-term, high-resolution imaging of layer 2/3 neurons in mouse visual cortex. This implied maximizing image quality, including dampening of brain pulsations, while maintaining animal and cortical health over multiple imaging sessions. These requirements were met by using orotracheal ventilation and a permanent glass window implanted over the visual cortex. The use of transgenic mice expressing variants of GFP in sparse neuronal subsets ensured stable, high levels of fluorescence.

Using this approach, we can consistently localize and image single layer 2/3 neurons at high-resolution, over multiple sessions spanning weeks or months. Individual dendritic spines as well as axonal boutons can be identified over the entire structure of these neurons, from the apical dendritic tuft down to the level of the soma and basal dendrites. This capability can thus be applied to follow morphological changes in the cortex of animals undergoing sensory manipulation.

The response of the visual cortex to focal retinal lesions provides a particularly interesting paradigm to study functional and morphological changes induced by alterations of the sensory input. Studies in cats and monkeys have shown that after induction of a retinal lesion, the initial cortical functional scotoma gradually regains visual responsiveness. Cortical cells in the deprived region develop new receptive fields, located in parts of the visual field just outside the lesioned region. Tracing studies have indicated that this functional plasticity involves the growth of intracortical horizontal connections.

By combining laser-induced focal retinal lesions, 2-photon microscopy, and optical imaging of intrinsic signals, we can chronically monitor the extent of the silenced cortical region as well as the morphological response accompanying the functional reorganization. From the methodological point of view, retinal lesions have the advantage of allowing for tight control over the position and spatial extent of the deprived region. Also, the dynamics of single cell morphology can be followed and compared between deprived, neighboring non-deprived and distant non-deprived regions, all within the visual cortex of the same mouse. Preliminary results show rapid reorganization of the axonal and dendritic level within the deprived region, a few days after induction of the

retinal lesion, indicating that microscopic morphological changes precede the macroscopic functional recovery.

Retinal expression of beta (B2)-crystallin and the heat shock protein Hsp27 after optic nerve axotomy and peripheral nervegraft-facilitated axon regeneration in adult rats 580

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Purpose: To investigate whether optic nerve (ON) axotomy and optic nerve replacement with a peripheral nerve (PN) graft induce protein expression changes within the retina due to neural de- and regeneration. Methods: Retinae obtained two weeks after retinal ganglion cell (RGC) regeneration into a PN graft were biochemically compared with retinae two weeks following ON axotomy and without treatment. The retinal proteins of each group were firstly subjected to 2d-electrophoresis, Coomassie-stain and subsequent MALDI-TOF-MS-analysis of differentially expressed spots. Secondly, the expression pattern of Hsp27 was examined by means of western blot and immunohistochemistry. Results: The proteomic analysis revealed β (B2)-crystallin to be expressed in untreated retinae, downregulated after ON axotomy and upregulated to almost basal levels in retinae following RGC regeneration. Hsp27 was not detectable in untreated retinae but induced by ON axotomy. A Hsp27-Neurofilament double labeling suggested the immunoreactivity to occur in surviving RGCs. However, Hsp27 was again downregulated following RGC regeneration into a PN graft. Conclusions: The extralenticular function of crystallins is largely unknown. These data may suggest a nonrefractive, metabolic role with respect to neural de- and regeneration. Induction of Hsp27 in axotomized RGCs likely mediates protection from apoptosis. The Hsp27-dependent survival pathway seems to be redundant when the injured RGCs contact Schwann´cells in the graft and are retrogradely supplied with neurotrophins.

Adjustment of displaced retinal ganglion cells to ocular growth in the chameleon (*Chamaeleo calyptratus*) 581

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INTRODUCTION & METHODS: Retinal ganglion cells (RGC) usually increase their dendritic field area as a response to postnatal retinal growth. The mechanisms that regulate the postnatal shape of dendritic arbors in the growing retina are not well understood. Quantitative studies suffer from the difficulty to label specific subpopulations of RGCs selectively including their dendritic processes. In this study, we selectively labelled by retrograde transport of dextran amines displaced retinal ganglion cells (DGC) that are known to project to the accessory optic system (AOS) with all dendrites in juvenile

and adult chameleons. These lizards display a number of unique visual specializations that have been studied extensively. The dimensions, shape, and retinal position of retrogradely labelled displaced ganglion cells were visualized in retinal wholemounts using a confocal laser scanning microscope (LSM 410-invert, Carl Zeiss GmbH, Jena, Germany). We screened the complete population of DGCs quantitatively for the effects of postnatal retinal growth on cell morphology, dendritic field coverage and arbor size.

RESULTS: We counted a total number of 2000 DGCs/retina in the adult chameleon eye and found that his number was already present in the freshly hatched animal with a retinal surface of approximately 1/3 of the adult size. With age, we observed a differential growth of the retina with an extension of surface mainly in the periphery. Accordingly, we found that the central area, defined by the physical landmarks of the fovea and the pecten, had an almost constant density of DGCs in juvenile and adult animals. Conversely, the density of peripheral DGCs was twice as high in juveniles than in the adults. The high density in the peripheral areas decreased with age corresponding to the differential growth of the retina. We found that the extend of dendritic coverage between neighboured cells was constant and unaffected by the changes in DGC density. To maintain the constancy of dendritic coverage in the growing retina, the size of the DGC dendritic trees was adjusted to the local cell density. The DGCs achieved this adjustment to retinal surface expansion mainly by dispersing their dendrites over a larger area. Literally, their initially condensed dendritic arbors blossomed out. To a minor extend, they also increased the length of interstitial dendritic segments.

CONCLUSION: The large extend of retinal growth in the chameleon eye together with a selective label of a particular cell population enabled us to study the mechanisms that regulate dendritic field size of RGCs with age.We found that AOS projecting retinal ganglion cells simply spread their dendrites over a larger area to adjust the area size of their dendritic arbors to retinal surface extension. This newly observed mechanism is different to previous observations in other non-mammalian retinae (goldfish, chick), where RGCs adjusted to retinal growth exclusively by a proportional extension of the total length of dendrites.

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582 Bifurcation between quasi-stationary cortical activity states alters the spatial and temporal distribution of response patterns

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Cortical activity under anaesthesia is not stationary but characterized by a sequence of discrete and stable states. Multi-unit neuronal activity is frequently analyzed using hidden Markov models that detect distinct states of neuronal activity. The activity, measured as neuronal firing rates, remains approximately stationary within each state and transitions between states occur quite abruptly. Similarly, the membrane potential recorded intracellularly from neostriatal spiny cells *in vivo* reveals two-state behaviour in the spontaneous activity where membrane potential shifts between two preferred levels.

These potential shifts correlate well with unit activity and to the slow electroencephalographic (EEG) rhythms that are typical of slow wave sleep and anaesthesia.

The cortical projection of the vibrissae in rodents is known as the 'barrel' cortex due to the cytoarchitecture surrounding the terminations of the thalamo-cortical axons. These barrels may be considered homologous to cortical microcircuits and provide certain advantages for experimentation. We used Current Source Density (CSD) profiles to map the location, extent and spread of neuronal activity throughout the cortical barrel following whisker stimulation.

Under urethane anaesthesia the mouse cortex also exhibits two quasi-stationary states of activity, quiet and brisk, and the multi unit activity (MUA) of cortical neurons to brief whisker displacements is also correlated with this activity. In order to de-interleave these alternate epochs we apply a Factor Analysis statistical method to the recorded evoked EEG potentials that provides an index vector. This index vector can be used prior to the calculation of the CSD profiles and, conveniently, may be used to de-multiplex the MUA activity. Data de-interleaved in this way exhibit significant differences in the evoked potentials; the CSD profiles; the population interactions and cross-correlation histograms collected during a particular activity epoch.

The CSD profiles show different temporal dynamics and provocatively, the locus of the initial activity in response to whisker stimulation moves away from layer IV during brisk epochs, towards layers II/III during the quiet epochs. Considering the static nature of the thalamo-cortical anatomy it is intriguing to speculate on how the locus of activity might actually move.

A set of neurons acting in concert is an instance of a neuronal assembly and if those assemblies are instanciated by selective synchronization of their distributed activities then we see the bifurcation from one cortical state to another provides a rapid and dynamic and mechanism to determine set inclusion and corresponding alterations in response behaviour.

Transient synchronization of cortical neurons using synthetic conductance injection

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The Binding Problem

It appears that sensory, motor and cognitive processes arise from parallel interactions between large populations of neurons. Binding is a mechanism of functionally linking this distributed activity into what we may call perceptual groupings. How relationships are established among one set of features to form a neural ensemble and how these ensembles are separated from one another is an interesting question. If the neuronal assemblies were defined by selective synchronization of their distributed activities this would provide a rapid and dynamic and mechanism to determine set inclusion. How is this rapid and transient synchronization achieved?

The effect of an inhibitory post-synaptic potential (IPSP) opening a chloride conductance is more pronounced the closer the post-synaptic membrane potential is to threshold because it is further from the chloride equilibrium potential. The greater the hyperpolarizing 'reset', the greater the delay in firing. Consequently, if an IPSP were 'broadcast' across a population of neurons, each subject to some individual depolarisation regime and whose membrane potentials were distributed according, then those neurons closest to threshold would be reset more than the less depolarized members of the set. To test this hypothesis required the injection of specific inhibitory conductances into pyramidal neurons at exact times. Note that these must be conductances; simple static current injection is not realistic and does not work!

A dual-channel dynamic clamp based on a digital signal processor that solved the ionic conductance equations in real-time was constructed (see poster DuBois & Engel) used to inject current into the neuron and re-sampled the membrane voltage for the next update. Using DIC-IR video microscopy with an *in vitro* preparation of rodent somatosensory cortex we injected a depolarizing current ramps into pyramidal cells and via a second patch electrode added inhibitory conductances at various phases of the depolarisation.

The hypothesis was confirmed with *in vitro* recordings from Layer V pyramidal cells in the rat somatosensory cortex. The IPSP magnitude increased with increasing depolarisation i.e. the closer the cell was to threshold and this introduces a congruent delay in firing despite the intrinsic membrane variability. With constant amplitude synthetic IPSP the firing times did not show the same consistent delay dependent on the actual membrane voltage. All other conditions being identical and the trials interleaved.

Dual recordings using the same experimental protocol but from cells undergoing slightly different depolarisation regimes were brought into transient synchrony.

The nonlinearity of the effect is crucial and relies on the voltage dependent chloride current being different in each cell that is 'reset' accordingly. The transient increase in synchrony (decrease in variance) across a set of neurons should translate into a powerful stimulus for any post-synaptic integrating neurons downstream. Such a decrease in variance is known as Fisher information.

584 The Impact of Higher-Order Correlations on Coincidence Distributions of Massively Parallel Data

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Following the hypothesis that assembly activity is expressed by temporal relations between discharges of participating neurons, neuronal responses need to be observed and analyzed with respect to temporal structure. With massively parallel recordings becoming available chances to observe the signature of assembly activity are increasing

[1]. Earlier we developed the unitary event analysis [2] to detect the presence of conspicuous spike coincidences in multiple single unit recordings. The presence of unitary events implies existence of correlations between the processes. However, deviation from expectation due to a correlation caused by a subgroup of neurons is not identified. For the detection of 'genuine' higher-order correlations we recently developed an approach that identifies coincidences which cannot be explained by a chance co-activation of lower order correlations [3].

The analysis for higher-order correlations, however, is computationally extremely demanding, and the effort increases combinatorially with the number of parallel processes. Thus, we aim at deriving a screening method, that allows a pre-analysis of the data, resulting in a reduction of the data set for further analysis extracting only the 'relevant' channels. Here we discuss an approach based on distributions of coincidence patterns, using numerical and analytical methods.

From data sets of N parallel spike trains we derive the probability distribution of the coincidence patterns observed across all N neurons. Such a set will result in max. 2^N individual coincidence patterns. For a first reduction of the data we pool coincidence counts of patterns of the same complexity, i.e. coincidences with the same number of spikes. By normalization we yield the probability distribution of patterns as a function of complexity, describing the full data set. In case of independent Poisson processes and identical spike probabilities p, the occurrence probabilities of the different patterns of one complexity are identical. In this case the distribution corresponds to a multinomial distribution with parameters N and p.

In case a specific subset of the neurons exhibits correlated firing of order m, the resulting coincidence distribution differs characteristically from the distribution in the independent case (same spike probabilities of the neurons). Interestingly, the distribution in the dependent case exceeds the original distribution not only at and above the complexity that was injected into the data, but also falls below independent pattern occurrence at lower complexities, and for even lower complexities again exceeds the pattern counts of the independent set. We provide an analytical model that fully explains these non-trivial features and also allows us to study the phenomenon as a function of various parameters (order of correlation, mixtures of different correlations, rate variations). The suitability of the finding as the basis of a screening method and the problem of considering coincidences with temporal jitter [4] is discussed.

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585 The relative contributions of prefrontal, posterior parietal, and inferior temporal cortices in extracting numerical information in monkeys.

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In a previous study, we reported that about a third of the neurons in monkey prefrontal cortex (PFC) signaled numerical information in visual displays. Whether the PFC, which constitutes the apex of the cognitive brain hierarchy, is indeed the origin of numerosity selectivity, or simply reflects highly-processed numerical information already present on presynaptic stages, remained an open question. In addition to PFC, we therefore recorded single-unit activity from behaving monkeys in the termination zones of the ventral visual ("what"-) pathway (anterior inferior temporal cortex, aITC) and the dorsal visual ("where"-) pathway (posterior parietal cortex) which are both reciprocally connected with the PFC.

Two monkeys viewed a sequence of two displays separated by a memory delay and were required to judge whether the displays contained the same small number of items (1 to 5 items). To prevent the monkeys from memorizing visual patterns to solve the task, the spatial arrangement and size of the items was randomized, and a given display was only shown once as sample stimulus. In addition, control stimuli were incorporated to control for changes in low-level visual features that may covary with changes in numerosity (such as total area, total circumference, dot density, linear of shape-like arrangement of the items, and item identity).

Each neuron was tested with pairwise combinations of all eight applied stimulus protocols. Neural activity was examined with a 2-factor ANOVA ($P < 0.01$) to find out whether a cell was selective for numerosity (1. factor: number of items), low-level visual features (2. factor: stimulus protocol), or both (factor interaction). A neuron was termed numerosity selective if it showed significant activation to numerosity alone.

About 25 % of the tested neurons in aITC responded to changes in numerosity. Most of these neurons, however, were also selective for low-level visual feature changes that covaried with numerosity; only about 5 % of aITC neurons responded purely to numerical changes in the displays.

Consistently low proportions of tested neurons showed numerosity selectivity in in various areas of the PPC (area 5 of the sup. parietal lobule, areas 5ip, VIP and LIP in the intra-parietal sulcus; areas 7a and 7b in the inf. parietal lobule). Depending on the area, only 1 to 10 % of parietal cells discharged as a function of numerosity. Changes of visual features in the displays had a neglectible impact on parietal neurons` firing rate.

Together these data indicate a central role of monkey PFC in abstract encoding of numerical information in visual displays. The proportion of numerosity selective neurons in PFC was three or more times higher than in any other presynaptic visual area. While most of the aITC neurons were still sensitive to visual pattern information, the vast majority of PFC neurons signaled pure numerical information irrespective of the exact physical appearance of the displays.

Distinct input-output characteristics are shown by three classes of spiny layer IV neurons in rat barrel cortex

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In sensory cortices, excitatory layer IV-spiny neurons are known to be involved in the initial stages of cortical information processing. Recently we found a cell type-specific pattern of intracortical synaptic inputs for spiny stellate vs. pyramidal neurons (incl. star pyramids; Schubert et al.; J.Neurosci. 15, in press). We characterized three classes of neurons in acute slices of rat somatosensory cortex (P19-21), by a combination of "blind" patch-clamp recording with biocytin-filled pipettes and quantitative morphological reconstructions: (i) spiny stellate (n=47), (ii) star pyramidal (n=22) and (iii) pyramidal (n=14) cells. Spiny stellate and star pyramidal cells showed either symmetric or asymmetric (polarized toward barrel center) dendritic trees. Star pyramidal and pyramidal cells both possessed an apical dendrite reaching into supragranular layers, however, they differed by the polarized organization of an apical versus a basal dendritic tree in pyramidal cells. The axons of these cells branched profusely in the vertical dimension and reached all cortical layers. Spiny stellate neurons showed an axonal arbor largely confined to their barrel-related column, whereas the axon of pyramidal cells spanned several columns. Star pyramidal cell axons were more similar to pyramidal cell axons, showing a tendency to send more branches into neighboring columns. Thus, layer IV-excitatory circuits are more differentiated than previously thought. Different cell types are preferentially involved in "local" versus "global" processing of tactile information.

Inference of Hand Movement Direction from Local Field Potentials in Monkey Motor Cortex I: Tuning Properties of Single Channels

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Many studies have shown that different movement parameters, like force or direction, can be related to the activity of single neurons in the motor cortex. Only few studies examined the local field potential (LFP), and quantitative data about the information

carried by the LFP is essentially missing. Given the difficulties to obtain stable multiple single-unit activity in human subjects, LFPs could be an advantageous additional signal for the control of neuronal motor prostheses.

Here and in the companion poster (1), we analyzed the directional tuning and decoding properties of LFPs recorded from the motor cortices of two rhesus monkeys. The monkeys were trained to perform a center-out hand movement task to eight directions arranged on a circle, while grasping a manipulandum with each of their hands (see (2) for details).

As shown in earlier work(2), LFPs typically exhibited a positive activity peak before the onset of movement (P1) and a negative peak around the onset of movement (N1), followed by a second positive peak during movement execution (P2) and a second negative peak around the end of the movement (N2). We found that many LFPs exhibited peak-dependent directional tuning. On average, the tuning curves of P2 were correlated negatively with both N1 and N2, while P1 and P2 were positively correlated. The shape of the tuning curves of many LFPs followed a cosine function ($r^2>0.5$ for 146/328 LFPs during movement execution). The preferred directions of the LFP tuning curves showed a non-uniform distribution with a bias towards the lateral movement axis. We also analysed the decoding power of single-trial LFP activity with respect to the target direction of the movement. For single channels, the accuracy of the decoding was similar to that of single cells and multi-unit spike activities (average 0.23). Contra- and ipsilateral recordings were likewise powerful decoding sources on average, but the decoding powers for ipsi- and contralateral movements of both single LFPs and single cells were uncorrelated. The LFP decoding power was time-dependent and correlated to the signal-to-noise ratio inherent in the LFP tuning curves. The activity around P1 yielded an average of one third of the total decoding power, but the activity around P2 yielded close to 90% of the total decoding power contained in the full time signal.

In summary, we have shown that the LFP possesses tuning properties with many aspects similar to that of single cells. Taking these results together with those of the companion poster(1), our results demonstrate the feasibility of the usage of the LFP as an important additional signal for the reconstruction of purposeful arm movements, and are therefore of considerable practical relevance for the development of neuronal motor prostheses.

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Spatiotemporal Dynamics of Thalamically Evoked Responses in the Barrel Cortex 588

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Sensory input in mammals is mainly conveyed via thalamic nuclei to the primary sensory areas of the cortex. We investigated the spatio-temporal dynamics in the rat barrel cortex after electrical stimulation of the thalamocortical pathway. Local field potentials (LFP) and spike activity were recorded simultaneously with planar microelectrode arrays in thalamocortical slices.

Presynaptic LFP activity (fiber volley, fv) generally spanned multiple barrels in layers IV to VI. Glutamatergic postsynaptic responses had column-like distributions starting 1-2ms after the first fiber volley in the input layers IV and Vb and at 5-30ms in layers II/III and VI. Spike activity was found locked to the first population EPSCs (pEPSC). In layers IV and Vb, a GABAergic potential directly followed the initial response. The decay-phase of this bicuculline sensitive component often led to rebound activity, manifesting as spike activity (mostly in layer IV) locked to the pEPSC. Paired pulse stimuli induced high-frequency LFP activity (ensemble activity, 15-70 Hz) across 2-3 barrels in layers II-Va, starting ~10-40ms after the second stimulus and lasting for >100ms. Shorter or longer intra-pair intervals reduced the probability of inducing ensemble activity (best at 20ms intra-pair and 20s inter-pair intervals). Ensemble activity was completely suppressed by blocking GABAergic transmission. Ensemble activity thus likely reflects local activation after release of synchronized inhibition.

In this pathway, thalamocortical synapses employ AMPA and kainate receptors and elicit cortical response patterns that can be modulated via ACh (Kimura et al., 1999, Eur J Neurosci 11:3597-3609) and NMDA receptors (Laaris et al., 2000, J Neurosci 20:1529-1537). They change the spread of thalamic activity in the cortex and the ratio between sensory input and ongoing cortical activity.

To examine the influence of neuromodulatory receptors on the structure of these LFPs, we first tested the role of NMDA receptors by withdrawing Mg^{2+} from the bath solution. This led to a reproducible change of the LFP in layers V and VI only. The time course of the response was delayed from 5 ms after the stimulus onward. The specific NMDA receptor blocker AP-5 reversed this effect reliably. Consequently, NMDA receptors appear to contribute to the modulation of the temporal structure of intracortical activity following thalamic stimulation.

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589 Inference of movement direction from local field potentials in monkey motor cortex II: Decoding from multiple channels

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Previous studies have shown that parameters of arm movements can be predicted from the spiking activity of a population of motor cortical neurons. Much less is known about the possible utilization of local field potentials (LFP) for the decoding of movement.

We compared the *decoding power* of multiple LFPs, multi-unit activity (MUA) and multiple single-unit activity (SUA) recorded in the motor cortex of two rhesus monkeys performing center-out arm movements (see [1] for details).

In the first part of our study (see companion poster, Rickert et al.) we could show that LFPs are tuned to the direction of arm movements, and that single LFPs carry essentially the same amount of information about movement direction as the spike trains of single cells. Here, we inferred movement direction from single-trial activity for an increasing number of electrodes. We found that

(1) the decoding power of MUAs and SUAs was roughly the same. The decoding power of LFPs was always higher than the decoding power of MUAs and SUAs, independent of the number of electrodes used. A relatively small number of LFP channels (48 combined from six different recording sessions) was sufficient to achieve more than 90 % decoding accuracy;

(2) the decoding power of LFPs increased with the number of recording electrodes, despite high signal and noise correlations between simultaneously recorded LFP channels;

(3) a time-resolved decoding procedure showed that LFPs and SUA carry information about the upcoming movement direction well (~100 ms) before the onset of movement. LFPs and SUA exhibited a similar temporal profile of decoding power, with the LFP being about 50 ms delayed in comparison to SUA;

(4) the highest decoding power was achieved by combining both signals, LFP and SUA, although the decoding power was then only slightly higher than for LFPs alone. We further investigated the degree of redundancy between LFPs and SUA by examining the relationship between the directional tuning curves of LFPs and single neurons recorded from the same electrodes. Our analysis revealed the presence of a weak, but significant correlation, both between the tuning of LFPs and single neurons, and between the tuning of single neurons alone.

Taken together, our results demonstrate the feasibility of using the LFP as an important additional signal for the reconstruction of purposeful arm movements, and are therefore

of practical relevance for the development of neuronal motor prostheses. Furthermore, the quality of directional tuning we found in motor cortical LFPs and the similarity of tuning between LFPs and SUA recorded from the same electrode suggest some degree of topographic mapping of tuning properties, as known to be present in visual cortical areas.

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Distributed Simulation of Large Biological Neural Networks 590

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In order to understand brain function, it is necessary to study the behaviour of large, highly connected neural networks. However, so far little research has focussed on networks with biologically realistic connectivity (in the order of 10^4 synapses per neuron). Such high connectivity can only be attained in networks of about 10^5 neurons, corresponding to approximately 1 mm^3 of cortical volume. A major barrier to such investigations is that the required simulations overtax the memory capacity of most easily available computers. Only distributed computing can provide us with the necessary memory resources. In addition, parallel computation enables long real time simulations to be completed on practical time scales. In the framework of our long term collaborative project NEST [1,2] to create a simulation environment for biological neural networks (BNN), we developed distributed simulation techniques using the widely available MPI library [3] specification. The resulting C++ simulation kernel distributes large, complex networks over several machines while fully maintaining the general design of the serial version. As with any parallel computing design, high efficiency can only be achieved by minimising the frequency and bulk of communication. The simulation is performed on a fixed time grid, however minimal communication frequency can be achieved by communicating not after each simulation time step, but after an interval defined by the minimum synaptic delay in the network. As in the serial kernel, communication between objects is triggered by the occurrence of an event in the source object. This scheme is highly efficient for spiking neurons. A further significant reduction of communication volume is obtained by using a distributed representation of neuronal target lists, ensuring that each synapse resides on the same machine as the corresponding postsynaptic neuron. We discuss the problem of random number generation in a parallel environment, and present a solution which ensures the reproducibility of results irrespective of machine number and platform. Further current problems include designing recording methods for a variety of possible architectures, and interfacing the simulation kernel with our interactive simulation language interpreter. As a benchmark, we simulate random and structured networks containing of the order of 10^5 neurons with realistic connectivity,

which are examples of our current research [4]. We quantitatively analyze the performance of the simulation environment on distributed systems (e.g. Linux clusters) and parallel (SMP) computers with respect to wall-clock time and speed-up. The benchmarks demonstrate the scalability of our approach with increasing numbers of neurons and processors. Partially funded by GIF and BIF.

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591 Latency Variability of Synchronous Spiking Emerging from Subthreshold Activation

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Electrophysiological experiments provide evidence for the existence of behaviorally relevant joint-spike events in cortical networks (e.g. [1]). A model system known to be able to generate high precision spike patterns is the synfire chain, which consists of several groups of neurons connected in a divergent/convergent feed-forward manner [2]. Under certain circumstances, the stimulation of one or more consecutive groups, e.g. by an injected current step, leads to the formation of highly synchronized spike volleys, which can propagate stably along the chain [3,4]. These pulse packets and the experimentally observed joint-spike events both have an internal temporal precision in the millisecond range. However, the experimental spike patterns occur only loosely time-locked to external stimuli or internal events [5,6].

We investigate to what extent the observed response jitter can be explained by a synfire chain embedded into a large background network of randomly spiking neurons. With the help of computer simulations we systematically study the dependence of this latency variability on features of the network and the stimulus. Focusing on subthreshold current stimuli with a sharp onset, we determine a lower bound for the magnitude of response jitter. Synchronous spiking activity can emerge from subthreshold stimulation at the price of large latency variability.

On the basis of theoretical considerations we can distinguish two sources of latency variability. First, the traveling speed of pulse packets is not deterministic. In different realizations pulse packets will therefore arrive at a given subsequent group at different times. This variability can be fully explained by a stochastic iterative mapping based on the deterministic description of pulse packet dynamics introduced earlier [4]. The second source of variability is the pulse packet initiation itself. Both the time and the location of the packet creation are stochastic quantities. Hence, even if the packet speed were constant the arrival times would differ.

Under suitable conditions latency variability can indeed exceed the temporal jitter within joint-spike events by an order of magnitude, even in chains with only a few neuron

groups. Our results on the variability of synchronous spiking provide important constraints for models of brain function based on interacting synfire chains.

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Distributed Computing for Neuroscience-Data Analysis 592

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The availability of multi-channel recordings from a variety of preparations enables the study of interactions between the channels and, thus, observation of the internal dynamics of brain processes. Advanced statistical methods are required to cope with the multi-dimensional non-stationary data generated by the brain. The emerging field of "Neuroinformatics$_{US}$" is dedicated to the development and application of appropriate tools. Massive data bases and the requirement of rapid data scanning in an interactive fashion imply the use of parallel computational methods. However, procedures are non-standard, subject to research, and typically need to be modified in every project. High-level programming language interpreters like Matlab [1] are used to enable flexible manipulation of the algorithms. In contrast, parallel computation requires programming on the C/C++ level and appropriate libraries. Here we present a framework for distributed computing suitable for interpreters like Matlab with no inherent support for parallelization. Although part of the computation is carried out in parallel the user interactively controls the software using a single instance of the interpreter. The central idea is to distribute standard operating system processes instead of parallelizing within a single user process or using a specific message passing library. The scheme is efficient provided computation is more expensive than communication. The first step is to isolate parts of the code that with respect to the data can be carried out in parallel. Next, these pieces of code are transformed to standalone scripts (*calc*) obtaining data from and writing results back to the file system. Typically the same operation needs to be carried out on a number of data segments (e.g. channels, time slices, conditions). The third step is to write a script *create* generating a standard makefile [2] which specifies the required calls to *calc* and their dependencies. The makefile ensures that *make* only returns when all tasks have been carried out. The final step is to write a control script performing a sequence of 4 actions: (1) serial preparatory work for data analysis, (2) call of *create*, (3) call of system command *make*, (4) serial post-processing (e.g. data collection). On multi-processor systems GNU *make* [2] can execute independent jobs in parallel. On distributed systems *ppmake* on top of PVM [3] takes over this role. We demonstrate our framework by a real world application, a parallel implementation of the Unitary Event Analysis [4] for multiple single-unit recordings. The performance is evaluated on diffe-

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593 The Spike Intensity Caused by Fast Supra-Threshold Input Transients

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Models of cortical neurons describing the instantaneous ability *f(t)* (or free intensity) to emit a spike are an important and mathematically convenient tool in the study of neural networks. Often the direct dependence on time can be removed and spike intensity *f* is linked to some variable *V* specifying the state of the neuron, *f(V(t))*. A prominent example for *V* is the membrane potential, but variables with units of current have also been considered (e.g. [1]). Different formalisms allowing for the fact that a neuron cannot emit spikes in arbitrarily short time intervals but exhibits refractoriness and adaptation have been discussed in the literature (e.g. [1-4]) and have been successfully applied to the study of synchronization dynamics of neural networks (e.g. [3,4]). Here we investigate the consistency of intensity models with integrate-and-fire models when exposed to fast supra-threshold transients in the input, assuming that the latter class of models represents a moderately realistic description of the spiking behavior of cortical neurons. Using simulations we obtain spike density functions (PSTHs) for an integrate-and-fire model subject to parameterized input transients. Numerically analyzing the simulated data, a generating free intensity *f(t)* is assigned to each PSTH. Comparison of the time courses of spike density and free intensity shows that the dominant mechanism concentrating response spikes on a ms time-scale and thereby potentially stabilizing spike synchronization is not refractoriness. In addition we find that the observed spike intensity cannot be explained by a *f(V)* relationship, where *V(t)* is the membrane potential excursion caused by the input. Assuming that membrane potential fluctuations are slow compared to the input transients, we analytically derive an expression showing that an instantaneous dependence of *f* on the state of the neuron can nevertheless be found. However, this state requires simultaneous characterization by *V* and its temporal derivative *d/dt V*. These findings suggest that the dominant role of refractoriness in some applications relying on intensity models may be due to the restriction of *f* to a function of *V* alone.

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The interplay between spike rate and correlation in neural feed-forward architectures

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Equilibrated activity states in models of cortical networks are often described in terms of spike rate or related measures. Experimentally, however, another standard measure to characterize cortical activity is the correlation between spike trains of simultaneously recorded neurons, gaining importance with the availability of multiple single-unit data. Recent theoretical studies have pointed out the impact of correlations between converging inputs on the spike rate of a single target neuron (e.g. [1]). Other studies [2,3] have emphasized the effect of correlations between the inputs of pairs of neurons on the output correlation. Here, we combine the characteristics of rate and correlation transmission to yield a model simultaneously describing the spatial propagation of both measures. Assuming stationarity and Poisson characteristics of all involved spike processes, we can exploit an analytical description of rate transmission. The pairwise correlation mapping is revealed by two-neuron simulations. The resulting input-output relationships are in agreement with earlier studies. In particular, we emphasize that the gain of pairwise correlation transmission is rather low for small and moderate input correlations. An important source of correlation in neural networks is that different neurons may share a certain part of their total input. An obvious example is a network where groups of neurons are connected in divergent/convergent feed-forward manner: the synfire chain [4]. It turns out that the input-output relations of spike rate and correlation can be used to construct a 2-dimensional iterative map describing the spatial spread of both variables along the chain. This dynamical system exhibits two attractors: a ground state, characterized by a low spike rate and almost zero correlation, and an excited state at high rates and intermediate correlation. The comparison of our model with the results of network simulations reveal an excellent agreement in the basin of attraction of the ground state. Outside, the system enters a synchronous mode characterized by spontaneously occurring spike packets traveling along the chain. In this regime higher-order correlations play a dominant role. However, the present study is restricted to pairwise correlations. Nevertheless, our approach provides insight into the mechanisms destabilizing the asynchronous ground state. We find that both group size and correlation gain act as bifurcation parameters. A high correlation gain as well as a large group size destroy the ground state. Since correlation gain is low in the relevant regime the ground state is robust with respect to correlation perturbations. In contrast, the excited state can be reached easily by a rate excursion. This explains the results of a former study [5] showing that the transition from the asynchronous into the synchronous regime is well described by a pure rate model.

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595 A psychophysical investigation of the detectability of electrical currents applied to the somatosensory barrel cortex of the awake rat

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Electrical stimulation in sensory cortices of awake subjects can evoke sensations. Here we investigate the effects of intracortical microstimulation (ICMS) in the barrel cortex of awake rats. Rats were trained to respond to an ICMS-train (train duration = 500 ms; frequency = 200 Hz; pulse length = 0.3 ms) by breaking a laser beam with their whiskers or their tongue and were rewarded if the stimulation event had been reported correctly. After termination of the experiments stimulation sites were confirmed by staining the barrel cortex for cytochrome C oxidase activity. Preliminary results indicate: (i) Rats can report ICMS in the barrel cortex. (ii) The lowest current thresholds correctly reported by the rats were < 30 microamps. (iii) Current thresholds were inhomogeneously distributed through the different layers and were substantially higher in supragranular layers (layer II/III) than in granular layers (layer IV). In addition we observed that electrical stimulation could interfere with the behavioral report of a simultaneously applied airpuff stimulus. The low current intensities and the inhomogeneous distribution of current thresholds suggest that the activation of small and specific groups of cortical neurons is sufficient for the rat's behavioral report. Thus, ICMS might be useful for a detailed mapping of the spatial distribution of induced electric cortical activity that gives rise to a behaviorally reportable sensation.

596 Morphology and physiology of L4 spiny neurones in developing rat barrel cortex

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The 'barrel field' in layer 4 of rodent somatosensory cortex is unique in that it preserves, at the cortical level, the structural organisation of the whisker hairs on the rodent's muzzle. In the rat, cortical barrels appear postnatally around P5-P6. We have studied the morphological and functional properties of layer 4 (L4) spiny neurones during and shortly after the process of barrel formation. Whole-cell recording from single and electrically coupled L4 spiny neurones of P6-P14 rats with simultaneous biocytin fillings were used. Neurones were subsequently fixed and processed and morphologically reconstructed using NEUROLUCIDA software.

Early during development virtually all L4 spiny neurones appear to have a pyramidal-like morphology: At P6, a large fraction of L4 spiny neurones has a prominent apical dendrite with or without a terminal tuft in layer 1 and symmetrically oriented basal

dendrites (90.4%). The apical dendrite is gradually lost with age: at P7-8, 78.5%, at P9-10 47.8% and after P14 only 17.9% of the neurones possess an apical dendrite which at this stage is always untufted and terminates already in layer 2/3. In contrast, an asymmetric orientation of dendritic branches of L4 spiny stellate cells – a prominent feature of mature barrel cortex – becomes only apparent with increasing age. Pyramidal-like L4 neurones have always symmetrically oriented basal dendrites and some immature non-pyramidal L4 spiny neurones display a symmetrical dendritic orientation. The axonal arbours of L4 spiny neurones are rather sparse at the beginning of barrel development and project often widely (more than 200 μm) into adjacent barrel columns. In the second and third postnatal week the axonal arbour becomes gradually more dense and confined to the barrel column and rarely extends more than 50-100 μm across its borders.

Paired electrophysiological recordings from two developing L4 spiny neurones showed the existence of non-rectifying electrotonic coupling probably mediated via dendritic gap junctions while synaptic coupling was extremely rare. The coupling coefficient of these gap junctions was on average 0.04±0.01 (n=6; mean±S.E.M.). In line with this intensive dye coupling of L4 spiny neurones was frequently observed. Dye coupling decreased with age (P6: 19.1±2.1 dye coupled neurones, P7-8: 11.2±1.0; P9-10: 6.5±1.1) and was no longer observed in the second week after barrel formation while synaptic coupling became clearly apparent.

Thus, during the first week after barrel formation L4 spiny neurones develop their mature morphology. This process is accompanied by a gradual loss in the possibility of these neurones to communicate via gap junctions. Gap junctions may not only allow electrical communication but also exchange of small metabolites which are involved in the structural changes during this period.

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Morphometry of the synaptic connection between layer 4 spiny neurones and layer 2/3 pyramidal cells in rat barrel cortex

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Columns are the functional units of the neocortex. In layer 4 of the rodent somatosensory cortex barrels are delineated cytoarchitectonically as barrels. We have studied the morphological and functional properties of pairs of synaptically coupled layer 4 (L4) spiny neurones and L2/3 pyramidal cells within a single column. Whole-cell recording with simultaneous intracellular biocytin fillings were performed. Neurones were subsequently fixed, processed and then morphologically reconstructed using a *camera lucida* system or NEUROLUCIDA software. To provide a quantitative description of the dendritic and axonal morphology of L4 spiny neurone - L2/3 pyramidal cell pairs within a

single column we developed a method to obtain two-dimensional (2D) maps of axonal and dendritic ?length density? from 2D reconstructions.

The projection field of the axons of L4 spiny neurones in layers 2/3, 4 and 5 has a width of 400-500 μm thereby defining an anatomical barrel column. In layer 2/3, the average axonal ?projection? field of a L4 spiny neurone is almost perfectly matches the dendritic ?receptive? field of the connected L2/3 pyramidal cell. Both form a column-restricted innervation domain extending vertically 300-400 mm and including mostly the basal dendrites. In the L4-to-L2/3 innervation domain a L4 spiny neurone contacts between 300-400 L2/3 pyramidal cells and forms about 30% of all synaptic contacts. The L2/3 pyramidal cell axon has two domains: a vertical one spanning all cortical layers, and a long-range horizontal domain in layers 2/3 (width 1100-1200 μm) and 5 (700-800 μm) projecting across column borders. Axonal collaterals establish synaptic contacts preferentially with dendritic spines and shafts of excitatory neurones and, less frequently, with GABAergic interneurones.

The results suggest that the extent of the vertical flow of excitation within a barrel column is determined by the columnar confinement of the L4-to-L2/3 innervation domain. When L4 spiny neurones are activated excitation will spread initially within a single barrel in layer 4 followed by or coincident with a vertical spread into layer 2/3. The translaminar transmission to layer 2/3 and the axonal projection domains of L2/3 pyramidal cells are the major determinants of the dynamic, multi-columnar cortical map in which a single whisker deflection is represented in the cortex.

598 Quantitative composition of synaptic responses in rat barrel cortex

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Cortical neurons receive several thousands of excitatory and inhibitory contacts from presynaptic neurons. It is not clear however, which fraction of those anatomically possible contacts is active during a sensory response. Here we determine the balance of excitation and inhibition of a cortical sensory response *in vivo* and estimate the number of active synapses in the respective response, based on *in vitro* data on the strength of cortical synapses. To this end principal whisker evoked responses in layer 2/3 pyramidal cells of rat barrel cortex were recorded in whole-cell configuration. In an ideal voltage clamp experiment it would be possible to directly estimate the contribution of excitation and inhibition from the current responses at different clamping potentials. However, the real experiment is subject to voltage clamp- and space clamp-error. To determine the composition of synaptic responses the experiments have to be complemented by computing the recorded cell. Thus, the recorded sensory responses were fit in the NEURON environment with a multicompartmental model based on the morphology of the recorded and reconstructed layer 2/3 neocortical neuron. Analysis of this model revealed, that the activity of 40 to 140 excitatory and 2 to 40 inhibitory synaptic terminals - this corresponds to 10 to 30 active excitatory unitary connections and 1 to 10 inhibitory unitary connections - reproduce the clamping behaviour of the recorded sensory responses

truthfully. It is concluded, that during a sensory response recorded in neurons of layer 2/3 of somatosensory cortex it is likely that only a very small portion of the anatomically possible synapses is active.

Subthreshold spatiotemporal receptive field properties of layer V neurons in somatosensory cortex 599

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Pyramidal neurons of layer V generate a major output of the primary somatosensory cortex. While action potential (AP) receptive fields of these cells have been studied by extracellular unit recordings, only limited information on the distribution of their subthreshold spatiotemporal receptive field properties is available. We performed *in vivo* whole cell recordings from layer V neurons of the primary somatosensory (barrel) cortex of urethane anesthetized rats (postnatal day 24-30). Whisker pad receptive fields were mapped for 45 neurons of these neurons 28 were subsequently identified by biocytin-DAB staining, providing anatomical and morphological information. These neurons had an average resting membrane potential of –73 ± 3 mV. Either multiple whisker deflections, by an air-puff, or single whisker deflections, by a piezo-electric device, caused predominantly excitatory postsynaptic potentials (EPSPs) in these cells. Although, the vast majority of the layer V postsynaptic potentials (PSPs) were depolarizing, some responses were marked by an initial inhibitory response that were always overwhelmed by an excitatory component. In addition to the principal whisker, layer V neurons could also respond to the single whisker deflection of many surround whiskers. The number of whiskers capable of generating a PSP (>1 mV) ranged from 1 to 10. The size of principal whisker PSPs (averaged over 20 trials) was on average ~6 mV but variable ranging from <1 mV to 12 mV. Stimulating the principle whiskers of the layer V cells generated on average 0.1 ± 0.2 APs. In addition, the majority of layer V cells responded to stimulation of ipsilateral whiskers with latencies longer and amplitudes smaller than those of the contralateral whisker responses. The current study demonstrates that layer V neurons, in comparison with neurons of layer IV and II/III previously studied in this laboratory, are unique in their relatively depolarized resting membrane potential, their relatively small whisker evoked EPSPs and the presence of a ipsilateral receptive field representation.

Movements Evoked by Intracellular Stimulation of Single Pyramidal Cells in Layer 5 and 6 of Rat Motor Cortex 600

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The relationship between AP discharge patterns of individual cortical neurons and motor behavior is a core interest of neurobiology. Extracellular stimulation and single unit recordings have demonstrated that activity of individual neurons in the primary motor

cortex is closely correlated with movement generation. Intracellular stimulation of identified single pyramidal neurons in the deep layers (L5 and L6) of vibrissae motor cortex in lightly anesthetized rats can evoke whisker movements in about 20% of all cells investigated. APs in layer 5 pyramids evoke rhythmic whisker deflections that were in phase from trial to trial. APs evoked in L6 pyramids generate rhythmic whisking whereby the phase relationship of movements in successive trials is variable. AP number strongly decreases latency to movement onset, whereas AP frequency strongly affects movement amplitude. The observation that APs in a single pyramidal neuron can generate motor movements in a cell type specific way suggests that generation of movements is controlled by sparse cortical AP activity.

601 A Model of Rapid Surface Detection in Primate Visual Cortex

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It has been shown that primates can perform image segmentation and subsequent classification using only 1 spike per processing stage [1,2].

In this contribution, we propose that surface detectors, responding to areas of homogeneous grey-level may support early image segmentation and facilitate the fast generation of an initial hypothesis about the input.

Surfaces can be defined as homogeneous image regions. To measure the homogeneity of an image patch, we use grey-level variance. For every pixel in the input image, the grey-level variance in a predefined patch, surrounding the pixel is calculated. Regions with low patch variance are then considered as surfaces.

We implemented this approach in a model network of spiking neurons, consisting of two layers. The first layer encodes the pixel grey-level in spike-latencies. Thus, homogeneous patches result in well synchronized pulse packets. Patch homogeneity is then detected by coincidence detectors in the second layer. If the grey-level variance in an image patch is small enough, a detector neuron responds.

We demonstrate the performance of the system using a set of real-world images. We show that even for images with a high degree of low-frequency clutter, the image is segmented into it's most salient components. Moreover, our model achieves fast image segmentation in a purely feed-forward manner without the need for lateral or feed-back connections. It uses only 1 spike per neuron and layer, therefore meeting the timing constraints set up in [1].

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Probabilistic model of spontaneous Purkinje cells firing in rats cerebellar slices: Application to the spike-sorting problem 602

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Spontaneous activities of Purkinje cells (PCs) were recorded in young rats cerebellar slices. Using multisite electrodes along the PCs layer, we could record from up to 8 linearly spaced sites (50 microns between sites). This allowed us to repetitively catch the activities of approximately 15 PCs simultaneously. We will show results of the spike sorting performed so far with our previous method (Pouzat *et al.*, 2002, *J.Neurosci.Meth.*, 122-143). Its main weak point is that it does not take into account spike waveform dynamics, *i.e.* the spike waveform's dependence on inter-spike intervals (ISIs), leading to a systematic misclassification of action potentials (APs) during bursts. Furthermore, cell-attached recordings of PCs provide us with spontaneous single unit discharges whose statistics can be fruitfully used as guide for the classification. In these recordings, PCs often fire bursts of 2 or 3 APs with dramatically decreasing amplitudes (*erg.* 50%). Such discharge statistics can be modeled with a Hidden Markov Chain (HMC). An algorithm able to fit HMC parameters from single unit data will be presented. This algorithm will be included in our new spike sorting method (Pouzat, Delescluse & Diebolt) which allows for a description of non-trivial neural discharge statistics as well as a description of spike waveform dynamics. This method will be tested on the presented data.

Anatomical maturation of the auditory cortex in the mustached bat 603

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The echolocation behaviour of the mustached bat develops during the first five postnatal weeks. There are many changes during this time period: the bats start to echolocate spontaneously in the second postnatal week, the frequency of the emitted calls shifts upward, the call duration lengthens and the cochlear filter mechanisms mature. Some auditory brainstem nuclei increase their volume and there is a progressive age related increase in axon diameter and number of myelinated fibres per unit area in all brainstem auditory centers.

This study analyses the anatomical maturation of the auditory cortex (AC) in Nissl- and fibre stained vibratome sections and Richardson stained semithin sections in specimens ranging in age from the first to the fifth postnatal week in comparison to adult bats.

Our results indicate that there are no significant age related differences in the thickness of the auditory cortex but there are pronounced changes in the myelinarchitecture. In the youngest specimens (first postnatal week) myelinated fibres are only found in the deep layers of the AC near the external capsule. They ascend vertically by only 200 mu m and their numbers decrease exponentially towards the surface of the AC.The following

development (second- third postnatal week) is characterised by an increase in myelinated fibres per unit area and an increasing vertical extension (about 400 mu m). These features are especially prominent in dorsal regions of the AC that likely include the FM/FM area that is known to evaluate target distance. At the end of the postnatal development (fifth postnatal week) the myelinated fibres occupy a larger part of the AC that also includes the region processing Doppler-shifted echoes (DSCF-area). Bats at the end of the postnatal development show all adultlike features in cochlea maturation and echolocation including Doppler-shift compensation and start to hunt insect prey on wings. The axon diameters remain small between the first and fifth postnatal week (averaging about 1 mu m). Adult bats have a mixture of myelined fibres in the AC which also comprises thicker fibres. The larger extension toward the cortex surface and the higher density of myelinated fibres in the upper cortical layers (II-IV) of adults compared to specimens of the fifth postnatal week indicates a prolonged maturation of the auditory cortex after the onset of flight and hunting behaviour.

604 Modulation of cortical excitability by neuronavigated transcranial magnetic stimulation (TMS) of the cerebellum - a pilot study

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Objectives Effects of rTMS can be propagated to other regions not directly stimulated, but functionally connected. Several studies could demonstrate that repetitive TMS over the cerebellum affects corticospinal excitability. To investigate whether rTMS over the cerebellum also alters inhibitory and excitatory phenomena within the motor cortex we used a conditioning-test paired pulse TMS paradigm. Methods In six healthy male volunteers cortical excitability was measured before and after cerebellar stimulation. We investigated the effects of medial and lateral cerebellar stimulation with high (10 Hz) and low (1Hz) frequency. A neuronavigational system adapted for TMS positioning enabled to monitore the exact position of the figure 8-shaped magnetic coil in relation to the target area. 1000 stimuli with a stimulus intensity of 120 % of the motor threshold were applied. Results Whereas stimulation of the medial cerebellum had no effects on motor cortex excitability, lateral cerebellar stimulation resulted in alteration of intracortical inhibition and intracortical facilitation. All subjects tolerated stimulation, but reported painful sensations in the stimulation area, especially with the high frequency condition. Conclusion These preliminary results demonstrate that rTMS over the cerebellum can modulate motor cortex excitability.

The cerebellar complex spike serves as the "teacher" reducing the motor error during saccadic learning 605

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Cerebellar Purkinje cells (P cells) generate two kinds of responses, driven by two classes of excitatory inputs: the simple spike (SS), with high firing rate (over 100 Hz) conveyed by the parallel fibers (PFs), mainly originating from the pontine nuclei and the complex spike (CS), with very low firing rate (~1-2 Hz), driven by the climbing fibers (CFs) originating from the inferior olive. Many theories of cerebellar motor learning (e.g. Marr, 1969 J Physiol 202: 437-70) propose that complex spikes provide the error signal essential for learning. In this view, the CFs serves as the "teacher" that encodes the deviation of the actual from the desired motor output. However, these theories do not specify how error information could be conveyed by the very low CS firing rate.

In this study we have investigated the role of CS during motor learning. We used saccadic adaptation as an example of motor learning. A targeting saccade can be changed by moving the target away from its original location while the saccade is being executed: a motor error is introduced. After many repetitions of such a target shift, the vector describing the first saccade to the target is adjusted such as to compensate for the target shift. With such a paradigm, we could introduce a motor error which is gradually reduced close to zero over several hundred trials. Hence, if the complex spike serves as the "teacher" reducing the motor error, the pattern of CS discharge should show a change which is correlated with the gradual reduction of motor error.

In order to test this hypothesis, we trained 2 macaque monkeys on saccadic eye movements. SSs and CSs from vermal (lobuli VI and VII) saccade related P cells were recorded before, during and after saccadic adaptation. Before motor learning, the CSs fired at random. During the saccadic burst of SSs, which is aligned with the saccadic eye movement, we observed a suppression of CSs, suggesting that the burst of SSs inhibits the CS response. In contrast, during learning the new saccade amplitude, the pattern of CS discharges changed: the CSs occurred preferentially in a period of 0 to 100 ms after the saccade-related burst of SSs. Furthermore, the probability of occurrence (Po) of CSs in this time window changed in the course of learning the new amplitude. In the early state of adaptation, Po was maximal (about 0.2) and was reduced close to zero at the end of saccadic learning with intermediate Po for intermediate stages of adaptation. The existence of such a monotonic relation between motor error and Po strongly suggests that the CS is the "teacher" contributing to reducing the motor error. The conjunction of SS and CS observed could be the basis of changes in synaptic efficacy , mediating motor learning as by suggested Marr 30 years ago.

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606 Lesions of lobuli VI and VII of the cerebellum cause oculomotor disturbances but do not impair visual motion perception

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The cerebellum has traditionally been understood as a structure devoted to motor control. This view has recently been challenged by imaging work and observations on cerebellar patients which imply the cerebellum in a wide range of non-motor functions, among others visual perception. For instance, patients suffering from cerebellar pathology have repeatedly been reported as being severely impaired on tasks of motion perception. While this deficit has been reported independently by various laboratories, using a large number of cerebellar patients, suffering from many different forms of cerebellar disease, as yet all attempts to define the functional principle, the cerebellum might contribute to motion perception, have failed. Moreover, the exact location of the minimal cerebellar lesion causing a motion perception deficit has been hard to delineate, because of the fact that the clinical lesions are usually relatively large and, moreover, not obeying anatomically defined parcellations of the cerebellum. What is clear, though, from the patient work is that the midline of the cerebellum needs to be affected by the lesion in order to disturb visual motion perception. Lobuli VI and VII (the "posterior vermis") and the dorsal paraflocculus, both located in the midline cerebellum, are the major recipients of visual input originating from parietooccipital cortex and, therefore, the cerebellar candidate structures, possibly contributing to visual motion processing. In order to test the possible involvement of these two parts of cerebellar cortex, we embarked on a study of the consequences of small, well-defined lesions of the rhesus monkey cerebellar cortex. In a first step, we lesioned the posterior vermis in two monkeys. The lesion in monkey R involved lobuli VI and VII, while the lesion in monkey B in addition included the rostral parts of lobulus VIII. The two monkeys were tested on a set of oculomotor paradigms, among others involving visually guided saccades and saccadic adaptation, known to depend on the integrity of the posterior vermis, on a task of visual motion perception and a task assessing the dependence of visual acuity on the allocation of spatial attention. As a consequence of the lesion, the monkeys showed hypometric saccades as well as reduced saccadic adaptation. On the other hand, visual acuity and the ability to enhance acuity by allocating spatial attention were unaffected by the lesion at least in monkey B (monkey R still being tested). Motion perception as assessed by defining the minimum percentage of coherently moving dots in large field random dot cinematograms required for the reliable perception of motion direction was not affected in monkey R and affected only mildly and transiently in monkey B (full recovery within 1 month). The weak effect of the lesion on motion perception suggests that lobuli VI and VII are not involved in the putative cerebellar contribution to visual motion perception, or, at least, that they are not essential. Future work will therefore have to tackle the more caudal vermal lobuli as well as the second major candidate structure, the dorsal paraflocculus.

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Altered fiber connections in human epileptic hippocampus - a dextran amine fluorescent tracer study

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Rationale: Beside altered intrinsic neuronal and glial properties network defects can cause epilepsy. We studied if neuron death and loss of afferent fibers associated with epilepsy lead to reactive recurrent sprouting and network derangement in the human hippocampus removed from patients with intractable epilepsy.

Methods: 54 acute slices (interface conditioning) of resected hippocampi with Ammon's Horn sclerosis (AHS) or associated lesions (Non-AHS) were labeled via an 80 µm dot-like lesion at different regions with fluorescent dextran amine tracer (MW 10,000), which was transported antero- and retrogradly and remained intracellularly.

Results: In AHS with severe gliosis only a few neurons and fibers were labeled in area CA1. Adjacent to severe gliosis (moderate AHS) and in Non-AHS, an increased labeling of pyramids remote from the loaded site was shown. In the dentate gyrus of AHS specimens a supragranular network of labeled fibers was present, corresponding to a positive Timm stain. Additionally, a significant number of fibers ran retrogradely from the hilus through the granular into the molecular layer of the dentate gyrus, mainly in specimens with moderate AHS or Non-AHS. Among these fibers were axons which showed an intense labeling and aberrant meandrous course within the entire dentate molecular layer, what only partly corresponds to the supragranular zinc labeling of aberrant mossy fibers.

Conclusions: Our study showed that there is reactive recurrent sprouting and network derangement in the CA1 region and the dentate gyrus of human epileptic hippocampus in specimens with moderate AHS and Non-AHS.

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Key words: network alteration, epilepsy, human hippocampus

608 Potassium release likely mediates spread of seizure like events under conditions of blocked chemical synaptic transmission.

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Lowering of $[Ca^{2+}]_o$ in rat brain slices *in vitro* induces spontaneous epileptiform activity (seizure like events, SLEs) in the absence of evoked synaptic transmission. These SLEs slowly spread across area CA1. Three mechanisms have been suggested to account for seizure propagation in this model: (i) ephaptic field interactions, (ii) electrical coupling through gap junctions, and (iii) K^+-mediated spread of epileptiform discharges. We studied the role of gap junctions between glial cells and neurones in seizure spread using pharmacological block of gap junctions with carbenoxolone and mice lacking the gap junction protein Cx43. Based on event-related statistics frequency of SLEs was found to be higher in Cx43-deficient mice compared with controls but velocity of seizure propagation did not differ between both groups. Carbenoxolone reduced the frequency of events in both Cx43-deficient and Wild-type animals ($p<0.05$) but did not change the spread velocity. Our results indicate that neither the gap junction protein Cx43 nor other gap junctions largely contribute to seizure spread. In addition, lowering of extracellular field potential gradients does not influence spread of SLEs under conditions of blocked synaptic transmission (Schuchmann et al.. 2002, J Neurophysiol. 87(6):2929-35). Therefore, it is likely that seizure spread is mediated by K^+-release from activated neurones which in turn excites neighbouring quiescent cells.

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Key words: Cx43 deficiency, hippocampus, nonsynaptic epileptogenesis

609 Long-Term Potentiation (LTP) in the Lateral Amygdala

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Activity-dependent changes in synaptic efficacy are believed to underlie the long-term functional modifications in brain circuits that allow behavior to be altered in response to experience. LTP at input synapses to the lateral nucleus of the amygdala (LA) is a candidate mechanism for memory storage during fear learning. Cellular mechanisms of LTP have been investigated mostly in coronal brain slices and field potentials or EPSPs were induced in the LA by stimulation of either thalamic afferents or by stimulation of the external capsule (EC). EC stimulation activates excitatory afferents from cortical structures, including the lateral entorhinal and temporal cortices, that course through the EC and synapse in the LA and basolateral nucleus of the amygdala. In our experiments we used a combined horizontal slice preparation, which preserved most of the connections to cortical areas and the hippocampus. The stimulation electrodes were located either in the EC or within the LA. The aim of the present study was the investigation of

the mechanisms of LTP induced either by weak θ burst stimulation (TBS) or by strong high frequency stimulation (HFS) by using the two different stimulation sites. Whereas a reliable LTP could be induced both by TBS and HFS by stimulation of the afferents running through the LA, TBS failed to induce LTP when EC was stimulated. HFS caused a comparable LTP in the LA by stimulation of the EC and the intranuclear afferents within the LA. We could show that LTP induced by TBS or HFS requires activation of the N-methyl-D-aspartate (NMDA) subclass of glutamate receptors. Whereas the NMDA receptor antagonist, APV, nearly completely abolished the induction of the TBS-induced LTP, the HFS-induced LTP was also dependent on calcium channels. Nifedipine, a L-type voltage-gated calcium channel (VGCC) antagonist, also blocked the HFS-induced LTP. In addition, we could show that HFS-induced LTP was dependent on kainate GluR5 receptors, because ATPA, the specific GluR5 agonist enhanced the LTP in the LA. LTP could be completely depotentiated when low frequency stimulation (LFS) was given 10 min after the induction of LTP. The reversal of LTP was incomplete, when LFS was applied 20 min after the induction of LTP. These findings indicate that HFS-induced LTP in the LA at least requires NMDARs as well as VGCCs. TBS-induced LTP seems to be only dependent on NMDARs. Further investigations with specific GluR5 receptor antagonists are needed to clarify the role of kainate receptors in the induction and mediation of LTP.

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The Amygdala is not the Hippocampus

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Two years ago an article written by Paul F. Chapman about the diversity of synaptic plasticity appeared in the journal "Nature Neuroscience". In this paper the statement was done that the amygdala is not the hippocampus because low frequency stimulation (LFS) of fibers in the external capsule produces synaptic enhancement in the basolateral nucleus of the amygdala (BLA) via kainate GluR5 receptors (Li et al., 2001). As known, LFS causes long-term depression (LTD) of synaptic activity in the hippocampus. In contrast to the results of Li et al. (2001) we observed that the stimulation of afferents running through the lateral nucleus of the amygdala (LA) as well as the stimulation of fibers in the external capsule caused LTD in the LA of picrotoxin-treated slices. Without the partial reduction of GABAergic inhibition by picrotoxin LFS only induced a short-term depression of synaptic activity. We found no significant differences in comparison to drug-free conditions, when we perfused the slices with ATPA, a specific GluR5 agonist. In both cases a comparable short-term depression was induced. In contrast, ATPA caused a significant enhancement of high-frequency stimulation (HSF) induced long-term potentiation (LTP) in the LA. Whereas Li et al. (2001) performed their experiments in the BLA of coronal brain slices, we used combined horizontal brain slices to investigate plasticity mechanisms in the LA. Therefore, two different amygdaloid nuclei were investigated in brain slices differing in their connectivity.

Nevertheless, we also found clear differences between the amygdala (LA) and the hippocampus (CA1 region of the hippocampus). First, HSF causes a weaker LTP in the CA1 region in comparison to θ burst stimulation (TBS), it was more effective in inducing LTP in the LA than TBS. Second, lateral amygdaloid neurons responded with a higher response probability that CA1 neurons, because paired pulse facilitation was significantly weaker than that in the CA1 region of the hippocampus. Third, there was a left-right hemispheric difference in the strength of LTP recorded in the LA. The mean percent of LTP recorded at the right hemisphere was significantly greater than that recorded at the left one. These hemispheric differences we found in Wistar rats as well as in Lister hooded rats. Such hemispheric differences were not obtained in the CA1 region of the hippocampus. These results show that there are functional differences between the single amygdaloid nuclei. Our results also confirm differences in plasticity mechanisms between the amygdala and hippocampus.

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611 Orexins/Hypocretins cause protein synthesis-dependent synaptic plasticity in the hippocampus

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Orexins /Hypocretins are novel hypothalamic neuropeptides linking behavioral state, appetite and neuroendocrine control. The full spectrum of functions and mechanisms of orexins, particularly with respect to higher brain functions such as cognition, emotion, learning and memory is not clear. Here, by using *in vitro* brain slice preparations from mice we show, that orexins by recruiting transcription- and protein synthesis-dependent mechanisms act as powerful modulators of synaptic plasticity in the Schaffer collateral pathway of the hippocampus, a brain structure implicated in many memory functions. In particular, Orexin A was found to cause a unique form of synaptic (meta)plasticity in the CA1 region (LTPOX), which requires activation of multiple plasticity-related kinases, such as PKA, MAPK, and ΠΙ3K. Importantly, LTPOX is also dependent on both transcription and protein synthesis, thereby priming macromolecular forms of synaptic plasticity induced by weak and otherwise ineffective stimuli. In spite of its early occurrence, the molecular signature of LTPOX is reminiscent to the late phase of LTP, a prominent cellular and molecular correlate of learning and memory in mammals. Thus, orexins not only regulate systemic arousal threshold and body weight but also threshold and weight of synaptic connectivity providing a molecular link between behavioral state, neuronal plasticity and memory functions.

Orexins/Hypocretins cause sharp wave- and θ-related synaptic plasticity in the hippocampus by orchestrating glutamatergic, noradrenergic and cholinergic signaling 612

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Orexin A and B, two recently identified hypothalamic neuropeptides, are implicated in the control of sleep-wake states, energy metabolism, and other homeostatic body functions. Their role in higher brain functions such as cognition, emotion, learning and memory is not clear. Here, by using *in vitro* brain slice preparations from mice, we provide substantial electrophysiological, pharmacological, morphological and molecular evidence that orexins cause synaptic plasticity in the hippocampus, a prominent cellular and molecular correlate of learning and memory in mammals. In particular, Orexin A causes a pathway-specific slow onset long-term potentiation (LTPOX) of basal synaptic transmission at Schaffer-collateral-CA1 but not mossy fiber-CA3 synapses. LTPOX has a presynaptic expression as suggested by PPF-experiments and requires sharp wave-concurrent CA-field burst activity bidirectionally related to long θ frequency stimulation, two behavioral state-dependent network oscillations. Moreover, consistent with the known behavioral state-dependent fluctuations of biogenic amines in the nervous system, LTPOX also requires co-activation of ionotropic and metabotropic glutamate, acetylcholine and noradrenaline receptors. In concert, these neurotransmitter systems activate multiple signaltransduction pathways and plasticity-related kinases such as CaMKII, PKC, PKA, MAPK, and ΠΙ3K. Together our data indicate, that orexins not only regulate behavioral state and homeostatic body functions but also hippocampal synaptic plasticity, providing a molecular prerequisite linking food, mood, sleep and memory.

The role of serotonin1A-receptors in the control of cocaine's behavioral and neurochemical effects 613

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Serotonin (5-HT)1A-receptors are found as somatodendritic autoreceptors in the raphe´ nuclei and as postsynaptic receptors in terminal regions of the 5-HT projections, such as the hippocampus or nucleus accumbens (Nac). Using in-vivo microdialysis in freely moving rats we found that the 5-HT1A-receptor antagonist, WAY 100635, can block cocaine-induced hyperlocomotion without affecting the dopamine increase in the Nac. Further experiments showed that cocaine causes an increase in extracellular 5-HT in the hippocampus parallel to locomotor activation. 5-HT1A-receptor antagonism with WAY 100635 potentiated the cocaine-induced 5-HT increase in the hippocampus and the Nac. Complementary, we found that systemic administration of the 5-HT1A-receptor agonist,

8-OH-DPAT, attenuated the cocaine-induced 5-HT increase in the hippocampus and the Nac, parallel to a potentiation of the cocaine-induced hyperlocomotion. In order to further investigate the role of hippocampal and Nac 5-HT1A-receptors we applied the 5-HT1A-receptor agonist, 8-OH-DPAT, locally via reversed dialysis. Local application of 8-OH-DPAT into the hippocampus attenuated the cocaine-induced 5-HT increase in the hippocampus, but also attenuated cocaine-induced hyperlocomotion. Local application of 8-OH-DPAT into the Nac potentiated cocaine-induced hyperlocomotion, but left the 5-HT increase in the Nac unaffected. The results of these experiments suggest an opposite role of different 5-HT1A-receptor populations in the behavioral effects of cocaine, namely an inhibitory role for hippocampal and a facilitatory role for Nac 5-HT1A-receptors. While hippocampal 5-HT1A-receptors are involved in the regulation of the local 5-HT response to cocaine, Nac 5-HT1A-receptors are not.

614 High frequency (200 Hz) oscillations in the basolateral amygdala and dorsal endopiriform nucleus of the behaving rat.

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The known repertoire of rhythms in the amygdala and paleocortex includes a range of oscillations from slow waves (<1 Hz) to fast γ (40 - 100 Hz). The dorsal endopiriform nucleus (EPN), laying lateral adjacent to the basolateral nucleus of the amygdala (BL) complex is known to generate high-frequency bursts and epileptiform population discharges *in vitro*. In the present study we show that in freely behaving rats the population discharge of neurons in the BL and the EPN is associated with field ~200 Hz oscillations in these structures. Microwire techniques were applied for recording single units and field activity from these stuctures and EEG from the dorsal or temporal CA1 subfields of the hippocampus. Units from both EPN and BL exhibited similar irregular firing patterns with bursts of 2-8 spikes (100-400 Hz). The mean firing rates in EPN were <1 Hz, whereas units in the BL fired in a range <1 Hz to 17 Hz . Neuronal activity in both BL and EPN was phase-locked with high-frequency field oscillations (HFO, ~200 Hz, 20-80 ms duration). Amygdaloid / EPN HFO displayed on average lower numbers of cycles and smaller amplitudes than hippocampal ripples. Neuronal firing and HFO in the BL and EPN were state dependent with a maximal occurrence during slow wave sleep (up to 20/min), being lower during waking and paradoxical sleep (down to 1/min). Averaged cross-correlograms between hippocampal ripples and EPN or BL units and field HFO did not reveal any synchrony. These data suggest common principles of temporal coding in hippocampus, BL and EPN in certain behavioural states via short scale population synchrony though they convey signals of different modalities.

Physiological and Morphological Characterization of Putative Cholinergic Interneurons of the Hippocampal Formation 615

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The large majority of hippocampal interneurons have been shown to utilize the inhibitory neurotransmitter γ-aminobutyric acid (GABA). There is, however, also evidence that a small population of interneurons express choline acetyltransferase (ChAT) but not glutamate decarboxylase (Frotscher et al., 2000). To facilitate the characterization of these supposedly cholinergic neurons, we generated transgenic mice in which the *in vivo* marker green fluorescent protein (GFP) was expressed under the control of the ChAT promoter. The GFP signal was present in cholinergic neurons in many subcortical regions including the basal ganglia, septum and brainstem motor nuclei. In the hippocampus only a small population of non-principal cells showed weak fluorescent labeling. These cells were primarily found in the dendritic layers of the CA1 area and near the granule cell layer of the dentate gyrus. By using videomicroscopy, whole-cell patch-clamp recordings were obtained from the GFP-labeled neurons in acute hippocampal slices. During recording the neurons fired at high frequency in response to depolarizing current pulses; action potentials were brief and were followed by a sequence of fast afterhyperpolarization (AHP), a depolarizing afterpotential and a medium AHP. When hyperpolarizing pulses were applied to the cells the responses showed a strong depolarizing "sag". Subsequent visualization of the biocytin-filled neurons revealed bipolar or multipolar dendritic organizations; the dendrites were beaded and mostly spine-free. The axon was primarily located among the apical dendrites of principal cells. In electron micrographs the axon was found to form symmetrical synapses on small caliber spiny dendrites. These properties are hallmarks of local circuit interneurons. To confirm the cholinergic phenotype of these neurons and to better understand their role in the hippocampal networks we need to further characterize their postsynaptic effects. (Supported by the DFG /SFB 505, TP C6/ and Novartis)

Immunohistochemical localization of metabotropic GABA receptor subtypes $GABA_BR1a/b$ and $GABA_BR2$ in the rat hippocampus. 616

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Metabotropic γ-aminobutyric acid receptors ($GABA_BRs$) play modulatory roles in both excitatory and inhibitory neurotransmissions in the hippocampus. To better understand the functions of $GABA_BRs$, the goal of this study was to elucidate the expression pattern

and the precise subcellular localization of $GABA_BR1a/b$ and $GABA_BR2$ subtypes in the rat hippocampus by using pre-embedding immunoperoxidase and immunogold methods. At the light microscopic level, immunoreactivity for both receptor subtypes was intense to moderate throughout the hippocampal formation and the patterns of R1a/b and R2 distribution overlapped well in CA3 field and dentate gyrus, but showed differences in CA1 field that exhibited less $GABA_B$ receptor-like immunoreactivity than CA3 area. Furthermore, intense $GABA_BR1a/b$ but not $GABA_BR2$ immunostaining was observed in somata of a subset of GABAergic interneurons of all hippocampal subregions. At the electron microscopic level, peroxidase reaction end-products for both receptor subtypes were predominantly present postsynaptically in spines and dendritic shafts of pyramidal cells as well as in interneuron dendrites in the dendritic fields of the CA areas. Immunogold particles for these $GABA_BRs$ were seen in clusters mainly along the extrasynaptic plasma membrane of pyramidal cell spines receiving excitatory synaptic input as well as in dendritic shafts and less frequently in dendrites of GABAergic interneurons. Occasionally, immunostaining for both R1a/b and R2 was observed in axon terminals making either asymmetrical or symmetrical synapses with spines and dendritic shafts, respectively. In the dentate molecular layer, peroxidase reaction end-products for both receptor subtypes were found postsynaptically. Immunogold particles were localized to the extrasynaptic membrane of dendritic spines of granule cells and, to a lesser extent, to presumed interneuron dendrites. These results suggest that GABAB receptors are involved in the regulation of both excitatory and inhibitory neurotransmissions acting at pre- and postsynaptic sites. (Supported by the DFG: SFB 505, TPB5).

617 Preservation of hippocampal neuron numbers in behaviorally characterized, aged tree shrews

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Numerous efforts have been made to answer the question whether neuronal loss in the hippocampal formation and entorhinal cortex can, at least in part, account for age-related decline in cognitive processes such as learning and memory. There is stereological evidence for a subfield-specific neuron loss in the hippocampal formation of aging humans. However, stereological analysis of hippocampal neuron numbers in young and aged rhesus monkeys revealed no hippocampal neuron loss [1]. Besides rhesus monkeys, which are considered a good animal model for neuropathological conditions, tree shrews (*Tupaia belangeri*) may constitute an interesting animal model for human aging [2]. Tree shrews are placed in the separate order Scandentia, and in many characteristics, they are closer to primates than are rodents. To test the cognitive abilities, we subjected 6 young (± 1 year of age) and 6 aged (4 - 9 years) tree shrews to a hole board test, in which both hippocampus-dependent and hippocampus-independent memory processes can be assessed [3]. After behavioral testing, we used the optical fractionator technique [4] to obtain stereological estimates of unilateral neuron numbers of the hippocampi of the experimental animals. Aged tree shrews showed a significantly impaired hippocampus-independent memory performance, whereas their hippocampus-dependent memory performance was not changed compared to young individuals. The stereological data revealed a preservation of hippocampal neuron numbers and no change in the volume of

the dentate gyrus granule cell layer and molecular layer. However, the volume of the dentate gyrus molecular layer correlated with the hippocampus-independent memory performance.

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3D MRI of mouse hippocampus in vivo: Contrast-enhancement using Mn^{2+} 618

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The hippocampal formation is involved in various brain disorders. Respective models of mutant mice have been developed. Recent MRI studies introduced manganese (Mn^{2+}) as a Ca^{2+}-analogue contrast agent [1,2]. The purpose of this *in vivo* 3D MRI study was to investigate the potential of subcutaneous and intrahippocampal administration of Mn^{2+} for a selective function-dependent enhancement of cerebral microstructures in behaving mice.

Subcutaneous $MnCl_2$: $MnCl_2$ (12mg/kg) within a glucose solution was administered subcutaneously to NMRI mice (n=5). 3D FLASH images (TR/TE=22/8.2ms, 30° flip angle, 120µm isotropic resolution) were acquired at 2.35 T (Bruker DBX) using a Helmholtz coil (100mm) and an elliptical surface coil (20mmx12mm) before as well as 6, 24, and 48h after Mn^{2+} administration. A pronounced enhancement was first (6h) seen in structures without a blood-brain barrier, such as the choroid plexus and intracranial endocrine tissue. Within 24h, Mn^{2+} contrast highlighted the olfactory bulb, inferior colliculi, cerebellum, and CA3 subfield of the hippocampal formation. In comparison with histologic data, enhanced structures most likely originate from the mossy fibers and adjacent pyramidal cell layer of the CA3 subfield, as well as from the dentate hilus with parts of the adjacent granule cell layer. The results suggests a region-specific neuronal uptake and axonal transport of Mn^{2+} related to brain function in behaving animals.

Intrahippocampal $MnCl_2$: After bilateral cannulation in a stereotactic holder adult mice (n=9) received $MnCl_2$ via a microinjector (0.2-1µl, 0.1-1M). After bilateral intrahippocampal injection of 0.2µl of $MnCl_2$ (0.2M) the fornix at the septal region was enhanced after 6h. This specific enhancement of the major extrinsic projection fibers evidently indicates neuronal uptake and axonal transport of Mn^{2+} by hippocampal pyramidal cells.
 In summary, the results suggest that Mn^{2+} enhanced 3D MRI adds new insights into function and connectivity of the hippocampal formation, which may contribute to a better understanding of memory and learning in normal/mutant mice.

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619 Social defeat produces lasting bidirectional reorganization of CA3 pyramidal neuron dendrites and synaptic plasticity

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Structural plasticity of the CA3 pyramidal neuron dendrites is considered to be an important outcome of the stress response when animals are chronically challenged over the course of several weeks. Here, we experimentally tested the difference in contribution of post-stress time and stressor recurrence. Adult male rats were exposed either to two social defeats followed by three weeks of single housing or to a repetitive series of social defeats lasting for three weeks. Whole-cell recording of kinetics and plasticity of the excitatory postsynaptic potentials (EPSPs) of the commissural-associational (C/A) CA3 synapses within *in vitro* hippocampal slices was performed in parallel with neurobiotin labelling to assess the structural properties of the CA3 pyramidal dendrites. Comparison of the synaptic responses showed that social defeat long-lastingly impaired LTP (98.4 ± 14.6 %, n = 7), with the repetitively defeated rats expressing a stronger reduction, even a reversal of the C/A EPSP amplitude (63.8 ± 8.9 %, n = 4). The quantitative morphometric analysis of labelled CA3 neurons of control, double defeated and repeatedly defeated animals indicated that defeat led to input-field specific remodelling. A double defeat followed by single housing decreased the dendritic length at the apical side of the CA3 pyramidal neuron (77 ± 10 % from control, n = 12), but in parallel increased in length and complexity of the basal dendrites (167 ± 12 %, n =12). Neurons from animals exposed to repeated defeat, possessed reduced apical dendritic surface and length only (69 ± 9 % from control, n = 8), in accordance to well established effects of chronic stress. These data demonstrate that even a short stressful experience produces long-lasting modifications at hippocampal neuron dendritic morphology and synaptic plasticity weeks after termination of the stressor. The additional effect of stressor repetition is an inhibition of remodelling of the basal dendrites, rather then being a necessary condition to induce dendritic atrophy at the apical side of the CA3 cell.

620 Auto/paracrine regulation of estrogen-induced synaptogenesis

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It has been shown that ovarian estrogens induce synaptogenesis in the CA1 region of rat hippocampus (for review see: McEwen 2002). Recently, we have demonstrated that estrogens are synthesized in hippocampal neurons. The differential expression of both genomic estrogen receptors (ERα and ERβ), which were taken as a parameter for estrogen responsiveness, furthermore provided evidence for an auto/paracrine loop of estrogen action (Prange et al. 2002). Here, we asked whether the amount of estrogens synthesized by hippocampal neurons is able to induce pre- and postsynaptic effects as has been

shown for peripheral or ovarian estrogens. To this end, we have studied the expression of presynaptic markers (synaptophysin, syntaxin, synaptotagmin I and IV) and studied spine formation as a postsynaptic parameter.

As expected, hippocampal neurons, cultivated for 7 days in a serum- and steroid-free medium expressed STAR and aromatase mRNA indicating the capabibilty of steroid biosynthesis. In the medium of these cultures considerable amounts of 17 β-estradiol were found, as revealed by radioimmunoassay. Supplementation of the medium with letrozol, an aromatase inhibitor, led to an almost complete inhibition of estrogen synthesis and in turn to a downregulation of ERα and an upregulation of ERβ (quantitative image analysis).

Confocal laser scanning microscopy revealed a dose dependent decrease of synaptophysin, syntaxin und synaptotagmin I und IV immunoreactivity due to letrozol treatment. The number of spines at apical dendrites of biocytin filled pyramidal cells was significantly reduced in the presence of letrozol. As a control, supplementation of the medium with 17 β-estradiol resulted in a significant upregulation of presynaptic markers and in an increase in the number of spines. Our results indicate that estrogen induced synaptogenesis functions in an auto- or paracrine manner (supported by DFG RU436/4-1).

Structural alterations in the limbic system of aged haploinsufficient trkB and/or trkC receptor knockout mice

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Neurotrophins act through a specific family of receptors, known as trk receptors. TrkB receptors are activated either by brain-derived neurotrophic factor (BDNF) or by neurotrophin 4/5 (NT4/5), whereas trkC receptors are activated mainly by neurotrophin 3 (NT3). Investigations concerning role of endogenous trkB and trkC receptors in the adult brain have been hampered by the early postnatal lethality of the corresponding homozygous knockout mice. We have used heterozygous trkB (+/-), trkC (+/-) and trkB/C (+/-)/(+/-), with an age of 21 to 23 month, to investigate possible morphological changes in limbic areas of these mice. Within the amygdala, neuronal densities were not altered, but there was an increase in the density of degenerated axonal fragments. In contrast, neuronal densities and total neuronal numbers in the hippocampus were altered in all three mutant models. Furthermore, an increase in the density of degenerated axonal fragments was noted in some hippocampal regions. Thus, the partial impairment in the receptor expression leads to structural disturbances. Increased densities of degenerated axonal fragments in the hippocampal formation and in the amygdala, may result from degeneration of both, intrinsic and extrinsic fiber systems. Our results indicate that endogenous neurotrophins are important factors for the maintenance of structural integrity of neurons during lifetime.

622 Distinct effects of enriched environmental housing conditions on hippocampal structures of aged rats

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Aging comprises a number of physiological modifications, including structural changes. Hippocampal information processing can deteriorate during normal aging in the absence of significant neuronal loss. These findings imply that a circuit-specific pattern of variability in the connectional organization of the hippocampus is coupled to individual differences in the cognitive outcome of normal aging. We addressed the question how enriched environment influences hippocampal structures in aged rats.

We used 19 hybrid Fischer 344* Brown Norway rats (FBNF1 rats) of following ages: 3 months (3 animals as controls), 36 months (16 animals divided into 8 animals housed in standard environment and and 8 animals housed under enriched living conditions during the last three months of their life) to ivestigate alterations of glial fibrillary acidic protein (GFAP), extracellular matrix protein as revealed by Wisteria floribunda agglutinin (WFA), neurofilaments (SMI-32 and MAP2), the presynaptic vesicle protein synaptophysin and the calcium-binding protein calretinin.

GFAP showed a complementary distribution pattern to WFA binding sites. With progressing age (12 – 36 months), a strong increase of gliosis occurred, whereas a concomitant, area-specific loss of WFA binding sites was found especially in CA1 region. The loss of extracellular matrix protein and the reduction of SMI-32 were in part reduced or prevented by housing the animals under enriched environmental conditions between 33-36 months of age. Synaptophysin was nearly lost in CA3 in the control rats. Calretinin which labeled the CA2 region exclusively displayed a small reduction only. Taken together we found area-specific both age-related and environment-dependent alterations in the hippocampus of aged rats.

623 Recovery of physiological function in dentate gyrus after global cerebral ischaemia

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Introduction: Histological evaluations after global ischaemia in rats showed death of CA1 pyramidal cells and preserved morphology of other parts of the hippocampal formation. With electrophysiological techniques we characterized neuronal populations which appear healthy by morphological criteria (dentate gyrus; toluidine blue/fuchsin acid) but seem to be functionally abnormal and, furthermore, try to find out whether we can induce regeneration of function.

Methods: Stimulating and recording electrodes were permanently implanted in adult Wistar rats for measuring population spike amplitude in the dentate gyrus after stimulation of perforant path. Recordings of baselines and I/O-curves were registered 1 day before induction of long term potentiation (LTP). 7-10 days after implantation LTP was induced in dentate gyrus by a tetanus of 200 Hz (10 bursts, 15 stimuli per burst, 0.2 msec stimulus duration, 10 sec stimulus interval) at a stimulus amplitude that was 40% of maximum. Population spikes were measured for 8 hrs after tetanization. One day after LTP global cerebral ischaemia was performed according to Dirnagl et al. (1993). For monitoring of blood pressure and checking of blood gases a catheter was inserted in tail artery. Via venous pooling blood pressure was reduced to 40 mmHg and simultaneously both common carotid arteries were clamped for 10 min. Body temperature was controlled and environmental temperature kept constant for 2 hrs post ischaemia. After 1,2 and 7 days baselines and I/O-curves were measured again. Once more tetanic stimulation was induced 10 days post ischaemia. Finally animals were decapitated 1 day post second tetatanic stimulation and after standard histological procedure cell death in hippocampus (CA1) und localization of electrodes were analysed.

Results: We found strong reduction of responses in dentate gyrus already on first day after ischaemia. In general, slope of I/O-curve was even more reduced on day 2, with many animals showing no response at all, even at maximal stimulation strength. Interestingly, it was possible to induce potentiation by tetanization on day 10 post ischaemia. Even in animals which showed no response at all in I/O curves we found long-lasting (at least 8hrs) responses after tetanic stimulation.

Conclusion: Even neurons which seem to be healthy by histological criteria can be functionally impaired after ischaemia. However, it seems that by tetanic stimulation we can induce partial recovery of function. These data together with findings on stem cell proliferation in the same area have significant implications for therapeutic strategies.

Cortical dendritic spine development is modulated by juvenile emotional and physical stress and 5-HT1A-receptor activation 624

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Previous studies demonstrated that the synaptic development of cortical brain regions is modulated by early postnatal cognitive, socio-emotional and pharmacological challenges. The aim of this study was to determine the influence of emotional and physical stress on the development of dendritic spines in somato-sensory cortex (SS) and limbic anterior cingulate cortex (ACd) of the trumpet-tailed rat Octodon degus. Furthermore, we attempted to identify a possible trophic role of the serotonergic system, in particular its 5HT1A-receptor, on postnatal synaptic development.

The densities of apical and basal dendritic spines of layer II/III pyramidal neurons in the ACd and the SS of 21 days old pups were quantified using the NeurolucidaTM system in the following animal groups: 1) social control (C): pups which lived undisturbed with their families, 2) parental separation (=emotional stress) (PS): pups which were separated individually from the parents for one our per day, 3) emotional stress plus physical

stress (=daily saline injection) (PS+S), 4) PS and daily injection (1.0 mg/kg) of the 5-HT1A antagonist Way-100,635 (PS+Way).

Our preliminary results for the somato-sensory cortex indicate that emotional and physical stress did not modulate the synaptic development (groups 1-3). Blockade of the 5-HT1A-receptor resulted in decreased apical spine density (19% compared to group 3), confirming observations in rats (Yan et al. 1997, Brain Res Dev Brain Res, 5, 98, 195). In contrast, spine development in the limbic anterior cingulate cortex is modulated by both, emotional and physical stress. Emotional stress induced a significant higher spine density in the anterior cingulate cortex (43%) for group 2 compared to control group 1. Physical stress combined with emotional stress (group 3) induced the opposite effect, a reduction (37% compared to group 2) of spine density on the apical dendrites of the ACd. Similar to the somato-sensory cortex, blockade of the 5-HT1A-receptors showed an decrease in apical spine density (15% compared to group 3).

These results indicate that stressful experience during early postnatal time windows induced by repeated parental separation or painful medical treatment significantly specifically modulates the synaptic development of limbic cortex (ACd), but not in somato-sensory cortex. In both cortical regions a trophical function of the 5-HT1A-receptor during postnatal synaptic development was demonstrated. The modulatory effects of such experience- and neurochemically induced changes of synaptic inputs in limbic cortical circuits may determine the cognitive and emotional capacity of these brain regions as well as their long-term behavioural outcome.

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625 Changes of parental behavior after acute an repeated separation from the offspring in the precocious species *Octodon degus*.

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Early social-emotional experience has a fundamental influence on the development of behavior and brain. Previous results in the trumpet-tailed rat (Octodon degus), a species which is like human babies born with functional visual and acoustic systems, pointed out that early postnatal deprivation leads to a variety of morphological changes including monoaminergic fiber systems, synaptic densities and alterations of GABAergic neurons and receptors in the limbic system (Helmeke et al, this meeting). So far, the behavioral outcome of these morphological changes was not analyzed in detail. Thus, the aim of this study was analyze behavioral changes in the parents as well as in their offspring after acute or repeated separation. Two group of animals were analysed at different time points during parental separation:a control group which remained undisturbed until postnatal day 21 and a group in which the pups were exposed to periodic parental deprivation (1 hour, postnatal day 1 until 21). The results indicate trends in some specific behaviors of both parents and the pups. In the pups of the separation group displayed increased locomotor activity and play behavior. Furthermore, change of behavioral elements which mediate social contacts (increased nosing) were observed.

Parental strategies also showed alterations in response to the separation procedure. The fathers of the separation group expressed more social contacts (nosing) but showed decreased huddling towards the other family members (mother and the pups) while the behavior of the mother of both experimental groups did not differ significantly. These results emphasize the importance and adaptivity of paternal care, which .most likely contributes to the developmental changes of brain and behavior, which is observed after repeated parental separation and other stressful emotional events.

"Synaptic tagging" and long-term depression in rat hippocampal slices *in vitro*. 626

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Studies were performed to investigate whether electrically-induced long-term depression (LTD) within rat hippocampal slices *in vitro* share any common cellular features with LTD in the intact animal, with particular emphasis being placed on mechanisms required for its late maintenance. In addition, we have investigated whether late-LTD reveals similar late-associative properties as long-term potentiation (LTP). Our initial studies have led to the development of stimulation protocols which are able to reliably produce different forms of LTD in hippocampal slices *in vitro*. Depending on the induction protocol applied, we were able to demonstrate a transient protein synthesis-independent early-LTD with a duration of up to 3-4 hours, together with a de novo protein synthesis-dependent late-LTD lasting for at least 8 hours. Thus, hippocampal slices *in vitro* show similar functional plasticity as intact freely moving animals if distinct care is taken during brain slice incubation. Furthermore, we were able to show input-specific LTD within the CA1-region, with expression shown only by those synapses specifically stimulated by a low-frequency protocol. In addition, preliminary data show similar functional, late-associative properties of LTD when compared with LTP. We present data demonstrating "synaptic tagging" during LTD in hippocampal CA1 *in vitro*.

Limbic interactions in the modulation of late phases of long-term potentiation in rat dentate gyrus *in vivo*. 627

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The prolonged maintenance of hippocampal long-term potentiation (LTP) requires heterosynaptic events during its induction. We have previously shown that stimulation of the basolateral nucleus of the amygdala (BLA) within a distinct time window can reinforce a transient early-LTP into a long-lasting late-LTP in the dentate gyrus in freely moving rats. We have shown that this reinforcement was dependent on ß-adrenergic and/or muscarinergic receptor activation and protein synthesis. However, since the BLA does not directly stimulate the granular cells of the dentate gyrus the question remained

by which inputs such heterosynaptic processes are triggered. We have now studied the possible involvement of the septal and/or entorhinal input to the hippocampus. Direct stimulation of the medial septal pathway or the perforant path 15 min after induction of early-LTP in the dentate gyrus are capable of reinforcing early- into late-LTP in a frequency-dependent manner. Interestingly septal reinforcement was dependent on ß-adrenergic but not muscarinergic receptor activation. In addition, preliminary results revealed a similar reinforcement of early- into late-LTP if the locus coeruleus was stimulated.

628 Possible antagonistic roles of TrkB and p75 neurotrophin receptors in modulating structural plasticity in the rodent hippocampus

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Functional and structural dynamics of neurons have an important role in the development and plasticity of neuronal circuits. While it is known that both dendritic spine number and dendritic arbor complexity can change during activity-dependent plastic processes (Yuste and Bonhoeffer, 2001) mechanisms and molecules involved in this form of structural plasticity remain largely unknown. Neurotrophins have been shown to modulate neuronal morphology as well as to support functional changes at synapses (McAllister et al., 1999). Specifically, BDNF, through its receptor TrkB, is a crucial component of activity-dependent strengthening of hippocampal synapses (e.g. Korte et al., 1995). In contrast, p75NTR as another receptor for BDNF has been reported to be involved in maintaining hippocampal long-term depression suggesting a possible role in synaptic weakening (Rösch et al., submitted).

The aim of our research is to analyze whether TrkB and p75NTR signaling systems are involved in morphological changes accompanying activity-dependent neuronal plasticity. Specifically, we explore whether p75NTR negatively modulates dendrite morphology, dendritic spine number and dynamics in hippocampal pyramidal neurons, while TrkB positively modulates them. Using the gene gun technology on organotypic hippocampal cultures, we transfected either wild type (WT) or p75NTR KO pyramidal cells, with various constructs. These include TrkB receptor, full-length p75NTR as well as intra- and extracellular truncated forms of it. Co-transfection with enhanced green fluorescent protein (GFP) allowed us to analyze the effect of neurotrophin receptor over-expression or deletion by confocal and time-lapse 2-photon microscopy.

Dendritic length and complexity were analyzed separately for basal and apical dendrites by using the Sholl analysis. In addition, spine density was measured in 3D stacks of basal, mid-apical and most-apical dendritic compartments. Compared to GFP expressing WT neurons, over-expression of p75NTR both in WT and p75NTR KO pyramidal neurons significantly reduced dendritic length as well as complexity in the basal and apical compartments equally. Moreover, spine density was drastically reduced in all dendritic compartments. In contrast, in GFP expressing p75NTR KO neurons total spine

density is increased by 100% and dendritic complexity is slightly greater than in GFP expressing WT neurons.

Our results strongly suggest that p75NTR may indeed play a role in mediating negative structural changes in mature hippocampal pyramidal neurons. Ongoing experiments using time-lapse 2-photon microscopy will be crucial in understanding the fate of the p75NTR over-expressing neurons and the dynamics of their phenotype. Furthermore, analysis of neurons over-expressing the intra and extra cellular truncated forms of p75NTR will clarify the mechanism of p75NTR action.

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Opposite Influence of Melatonin to the Synaptic Transmission in Rat Hippocampal Slices During the Circadian Cycle 629

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The aim of the experiments was to analyse circadian effects of melatonin on the transmission at the Schaffer collateral/CA1 synapse.

Rats were entrained to a 12/12 hrs LD cycle (ZT0 = lights on). Hippo-campal slices were prepared at ZT 3 (day group; n=24) and ZT 20 (night group; n=12) and analysed in a submerged recording chamber. After stimulation of the Schaffer collaterals field potentials (fEPSP) were recorded from str. radiatum of the CA1-region.

Melatonin (10 nmol/l) increased the slope of fEPSP in slices of the day group more than 2 times of the initial level. In contrast to this, melatonin decreased the slope of fEPSP in slices of the night group to 50% of the initial level. The enhancing effect of melatonin was suppressed by admini-stration of the specific antagonist luzindole (100 nmol/l). In paired pulse inhibition (PPI) experiments melatonin exerts opposite effects: a decrease in slices of the day group and an increase in slices of the night group.

The experiments suggest that the action of melatonin on the synaptic efficacy in CA1 is mediated by GABAergic interneurons.

Postnatal maternal separation up regulates BDNF mRNA in the hippocampus of BALB/cByJ, but not C57BL/6J or DBA/2J mice. 630

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Adult sequelae to early adverse life experiences are hypothesized to be substantially influenced by the genetic makeup of the individual. The present study was undertaken to

better understand the potential developmental consequences of early postnatal maternal separation on the hippocampus, specifically, the expression of serotonin $(5\text{-HT})_{1A}$ and brain derived neurotrophic factor (BDNF) in the hippocampal subfields. C57BL/6J (C57), DBA/2J (DBA), and BALB/cByJ (BALB) mice were exposed to 15 or 180 minutes of separation from their dam from postnatal days (PND) 2 through 14. *In situ* hybridization was performed on the brains of adult animals. Results show that, in BALB mice, 180 minutes of maternal separation increased levels of BDNF mRNA in each of the hippocampal subfields examined. 5-HT_{1A} mRNA was found to be increased in the dentate gyrus, again, only in BALB mice. Surprisingly, no measurements of corticosterone (CORT), glucocorticoid receptor (GR), or mineralocorticoid receptor (MR) mRNA were found to be altered in BALB/cByJ mice, indicating that the changes in BDNF and 5-HT_{1A} are not maintained by the actions of CORT. These data suggest an up regulation of the synergistic actions of 5-HT_{1A} and BDNF in establishing serotonergic connections in the hippocampus. Our results further suggest that BDNF and 5-HT_{1A} are potential mediators of the interaction of genetic and environmental factors that produces an adult phenotype.

631 Limbic predictors of rTMS effects in patients with affective disorder as measured by ECD-SPECT

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Objectives: Although recently published studies suggest that repetitive transcranial magnetic stimulation (rTMS) may have significant antidepressant properties, there is a substantial variability in response. Thus, we investigated whether cerebral activity of different brain areas or clinical data can predict successful outcome to rTMS. Methods: 20 patients with major depression were treated over two weeks with high frequency (10Hz) rTMS over the left dorsolateral prefrontal cortex as an add-on treatment to antidepressant medication. ECD-Single photon emission computed tomographay (SPECT) imaging was performed before and after rTMS. Clinical data were obtained at baseline and at the end of the study by trained psychiatrists blind to the mode of treatment. Results: Using a multivariate regression model with simultaneous evaluation of the relative impact of a-priori chosen potential factors influencing treatment outcome, only two variables, the pretreatment anterior cingulate activity and the former response to antidepressant agents proved significant. Patients showing a better response to rTMS had a significant higher pretreatment anterior cingulate activity than those responding less well. Conclusions: Pretreatment anterior cingulate activity seems to be a useful prognostic marker of rTMS treatment response, which is in line with other treatment strategies, like sleep deprivation, electroconvulsive therapy or antidepressant medication.

Temporary inactivation of the medial amygdala blocks freezing in rats induced by trimethylthiazoline, a component of fox feces 632

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Naïve laboratory rats that are exposed to the odor 2,5-dihydro-2,4,5-trimethylthiazoline (TMT, a component of fox feces) show freezing behavior, a known behavioral indicator of fear and anxiety in rodents (Wallace & Rosen , 2000). Our lab had previously demonstrated that the bed nucleus of the stria terminalis (BNST) rather than the lateral and central nucleus of the amygdala is involved in TMT-induced freezing (Fendt et al., 2003). However, since the ventral part of the medial amygdala is known to receive strong olfactory input, the present study focused on the effect of temporary inactivation of this nucleus during TMT-exposure.

We accomplished temporary inactivation by local injections of muscimol (2.2 nmol/0.25 µl), a $GABA_A$ receptor agonist, into the area of interest. Temporary inactivation of the medial nucleus of the amygdala blocked TMT-induced freezing. Preliminary data reveals that this effect was not caused by an enhancement of motor activity following the lesion.

We are currently testing the effects of muscimol injections into the basolateral part of the amygdala on TMT-induced freezing. The basolateral nucleus of the amygdala is relevant for contextual fear-conditioning. In addition, we plan to examine c-fos expression in the different amygdaloid subnuclei after TMT presentation.

The present study shows that the various subnuclei of the amygdala play different roles in the mediation of TMT-induced freezing. The medial nucleus (as well as the BNST) is necessary for TMT-induced freezing, whereas the central and lateral (and probably the basolateral) nucleus of the amygdala seem not to be involved in this kind of fear behavior.

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Movement-correlated neuronal activity in the hippocampus: Evidence for motor representation in the hippocampal formation 633

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In the hippocampus of freely moving rats, neurons have been described that fire predominately in a particular area that the animal passes through while exploring a maze (so-

called 'Place cells'). Other types of neurons can be found that fire in correlation with the movements of the animal. Most of these cells are direction-specific and fire along the whole length of maze arms. Such cells do not fulfill the definition of place cells and appear to code more than spatial information. We postulate that spatial information is linked with egocentric motor information in the context of the animal's behaviour.

To test this theory, animals were trained to learn a stereotypic motor task in a figure-8-shaped maze. 16 electrodes were implanted in the hippocampus of animals. Single neurons were isolated by use of a tetrode configuration (Axona, UK). Recording from 4 Long-Evans rats, 114 cells were isolated in 18 runs. 23 out of 45 cells that fired in the central arm fired in correlation with the animals' movements. 20 out of 45 cells were classified as place cells. Interestingly, over the course of training, an increasing number of cells were found that fired in a movement-correlated way (linear regression, $p<0.001$), suggesting that the association with movements has to be learned over time.

It is proposed that these cells associate egocentric motor information with allocentric spatial information to form behavioural programs that enable the animal to perform the task.

In an additional experiment, the injection of 0.1mg/kg Haloperidol *i.p.* strongly reduced the spatial tuning of both place cells and movement cells (23 cells; ANOVA $p<0.001$). This result suggests that information transfer from brain areas such as the basal ganglia that are dependent on dopaminergic activation exert stabilising influence on hippocampal cell activity. This finding supports the hypothesis that motor information that is coded in the motor system is transferred to the hippocampus. Further experiments are planned in which dopamine antagonists are injected locally into the dorsal striatum while neurons in the hippocampus are recorded from.

634 Specificity of inhibitory signalling in the amygdala

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Behaviours mediated by the amygdala, especially fear, are the result of a delicate balance between excitation and inhibition. Substances that influence inhibitory synaptic transmission have profound effects on these behaviours. Benzodiazepines, which potentiate inhibitory synaptic transmission through $GABA_A$ receptors, reduce the acquisition and expression of fear. $GABA_A$ receptors are hetero-pentameric chloride channels that occur in a wide variety of configurations; most frequently they contain two α, two β and one γ subunit. Channels containing either $\alpha1$, $\alpha2$, $\alpha3$, or $\alpha5$ subunits are benzodiazepine sensitive. A single amino acid in these subunits controls benzodiazepine binding. Knock-in mice with point mutations at the relevant site that render the receptor benzodiazepine insensitive have been made for all four α subunits. With these mice it has been shown that $\alpha2$ subunit containing $GABA_A$ receptors exclusively mediate the anxiolytic effects of benzodiazepines (1).

We therefore investigated $GABA_A$ receptor mediated synaptic transmission in the amygdala at a morphological and functional level in order to find a possible correlate for

this specificity in GABAergic signalling. Whole-cell patch clamp recordings were obtained from principal cells in the lateral, basolateral and central nuclei of the amygdala and monosynaptic inhibitory postsynaptic currents (IPSCs) were evoked with extracellular stimulus electrodes. The effect of benzodiazepine application on these IPSCs was then studied in point-mutated and wild type control mice. In the lateral and basolateral amygdala a significant reduction of the benzodiazepine effect was observed only in mice with both $\alpha 1$ and $\alpha 2$ point mutations, whereas in the central amygdala a point mutation in the $\alpha 2$ subunit alone had a significant effect. These results corresponded with antibody stainings, which showed a high density of $\alpha 2$ subunit expression and a very low density of $\alpha 1$ subunit expression in the central amygdala. The expression levels in the lateral amygdala were more evenly distributed.

There is a subunit dependent specificity in $GABA_A$ receptor distribution in the amygdala that can be demonstrated both at the morphological and functional level. Such specific receptor distribution may underlie the observed selective behavioural effects of benzodiazepines in the point-mutated mice.

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Honeybees generalize shape features acquired through image motion

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In an earlier study (Lehrer and Campan 2002) we demonstrated that bees discriminate among closed shapes producing either luminance contrast or motion contrast against their background. Edge parameters proved to be crucial in this task (Campan and Lehrer 2002). In the present study we examine the bee's performance in using shape parameters extracted from image motion for discriminating novel shapes.

Bees were trained with two black-and-white randomly patterned shapes, a triangle and a disc, offered simultaneously 5 cm in front of a similarly patterned background. Thus, the shapes produced motion contrast, but no luminance contrast against the background. In two separate sets of experiments, either the trainge or the disc offered the bee a reward of sugar solution on every visit, whereas the alternative shape did not. The trained bees were then presented with different pairs of novel shapes.

Bees trained to the triangle preferred it to the disc in 85.3% of their visits (n = 3686). A pair of shapes displaying just the contours of the two shapes rendered 86.7% correct choices (n = 208). Shapes displaying only of the upper half of the contours, the lower half, or other portions of the contours, were again discriminated well (81.2%, n = 192; 74.5%, n = 216, and 68.2%, n = 173, respectively). Generalization capacity is evident from the finding that the patterned (learned) triangle was strongly preferred when tested

against each of the modified triangles. Discrimination was significant even when homogenously black shapes were presented against the patterned background (66.5%, n = 173), or against a white background (60.9%, n = 202), in which case relative motion was absent. Using blue, yellow, or green pairs of shapes placed against the patterned background, preference of the triangle was 78.1 % (n = 283), 72.2 (n = 338) and 85.2 (n = 284), respectively. This performance is probably based on occlusion at edges, because discrimination broke down when there was no distance between the shapes and the background.

In the reciprocal training experiment, with the disc rewarding and the triangle not, similar results were obtained. From these and further results (Campan and Lehrer, in prep.) we conclude that bees generalize spatial cues detectable through relative motion (i) to novel shapes displaying only the contours of the training shapes, or parts thereof (ii) to homogenously black or coloured shapes, (iii) to shapes visible by luminance contrast rather than by motion contrast, and (iv) to shapes visible by occlusion rather than by motion parallax.

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636 Group I mGluRs regulate the expression of the neuronal calcium sensor protein VILIP-1 *in vitro* and *in vivo*: Possible implications for mGluR-dependent hippocampal plasticity?

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Metabotropic glutamate (mGlu) receptors are involved in several forms of synaptic plasticity such as LTP and LTD in the rat hippocampus. Furthermore, agonists which activate group I mGlu receptors induce slow-onset potentiation without prior tetanization in the hippocampal area CA1. Activation of group I mGlu receptors induces protein-synthesis which may contribute to mGlu receptor-dependent forms of long-term plasticity. In order to further characterize the association between mGlu receptors, LTP and protein expression, we examined whether application of DHPG *in vivo* at concentrations known to induce protein synthesis and slow-onset potentiation may lead to an altered expression of calcium-binding proteins which are widely considered to comprise key elements for synaptic plasticity. Neuronal calcium sensor (NCS) proteins influence various neuronal signalling pathways which are known to be relevant for learning and memory processes. Preliminary results have indicated increased levels of the NCS-protein VILIP-1 mRNA 24 and 48h following LTP induction. These results are in line with data from the literature which show that NMDA receptors also show increased expression level 48h following LTP induction and may thus contribute to the persistence

of LTP. Therefore, we investigated whether NCS proteins are associated with glutamate (group I mGlu) receptor-mediated plasticity in the dentate gyrus (DG) *in vivo*.

Application of either the group I and II mGlu agonist (1S,3R)-1-aminocyclopentane-1,3-dicarboxylate (ACPD) or the selective group I agonist (R,S)-3,5-dihydroxyphenylglycine (DHPG) resulted in slow-onset potentiation in the DG of adult rats. In hippocampal cell cultures both agonists elicited an enhanced expression of VILIP-1. *In situ*-hybridization revealed strong hippocampal expression of VILIP-1 and intracerebral application of DHPG to adult rats significantly enhanced hippocampal VILIP-1 expression. The DHPG effects in both, hippocampal cultures and *in vivo*, were prevented by the group I mGlu receptor antagonist 4CPG. Similar observations were made for other NCS-proteins.

Calcium sensor proteins thus appear to be regulated by mGlu receptors in an activity-dependent manner. A specific role for group I mGlu receptors is evident. Furthermore, the sensor proteins may function as molecular switches for the long-term regulation of synaptic plasticity.

Anatomy and odour-induced calcium activity in the mushroom bodies of honeybee (*Apis mellifera*) brain using 2-photon microscopy. 637

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The mushroom bodies (MBs) are the parts of insect brain that represent higher-order centers performing integration of olfactory, visual and mechanosensory information and are involved in neural plasticity underlying associative olfactory learning. MBs receive inputs from the first-order olfactory neuropil, the antennal lobe via project neurons. The sensory inputs in MBs are sorted out in the calyx regions: olfactory in lip region, visual in collar region and mechanosensory – predominantly in the region of basal ring. It is supposed that the synapses between MB neurons (Kenyon cells) and projection neurons are the substrate for learning-induced plasticity, but it is not known how olfactory information is represented in the MB on the level of single Kenyon cells and how this would change during repeated stimuli. Addressing this question we used Ca^{2+} fluorescence microscopy together with 2-photon laser scanning microscopy (2PLSM) to see how the odour information is processed by somata and dendrites of Kenyon cells and whether computation of olfactory information changes during stimulus repetition.

To visualize both the Kenyon cells morphology and activity we used the functional Ca^{2+} sensor FURA-2-dextran, which was delivered into the cells using the back-filling method by injecting the dye into one of the output region of MB, the α lobe. The selectively stained Kenyon cells were recorded optically *in vivo*.

Raw fluorescence of FURA-2 allowed us to visualize the morphology of MB and the Kenyon cells. 2PLSM allows restricting the collected information to a narrow focal

plane, enabling us to make a 3D reconstruction of Kenyon cells at high spatial resolution using Amira software.

Using FURA as a functional dye, we measured the changes of Ca^{2+} concentrations evoked by odour stimuli. The signal appeared to be restricted to particular morphological structures (separate spots in dendrites and cell bodies). Odour stimuli induced reproducible activity patterns, which appeared approximaltely 200 ms after the stimulus onset, reached the maximum within 500 ms and lasted for 2-3 secs, depending upon the duration of odour presentation. Some cells showed also odour-concentration dependent activity. At higher concentrations we saw phasic-tonic responses with smaller off-responses.

Since the fluorescence signals, as detected with the 2PSLM, are rather strong and spatially highly separated it will be possible to analyze olfactory coding at high spatial (1 mkm^3) and temporal (about 2-3 ms) scale.

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638 Essential role of the mushroom bodies for memory retrieval after olfactory learning of honeybees

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The mushroom bodies (MBs) play a central role in computing environmental information in insects. They get input from almost all sensory organs, including visual and olfactory information. Therefore, the MBs play a major role in olfactory learning and memory. Honeybees, Apis mellifera, can associate an originally neutral odour (conditioned stimulus, CS) with a sucrose solution reward (unconditioned stimulus, US). Forward pairings of a CS and a US enable the CS to release the proboscis extension response (PER) in consecutive memory tests. Bees can also be conditioned differentially: They learn to react to a rewarded CS (CS+) and not to a non-rewarded one (CS-). Using unilateral blockade of neural activity of the MBs by tetrodotoxin (TTX) we tested whether honeybees need their MBs to retrieve learned information about odours. Bees were trained with differential conditioning tasks. We tested three cases of differential conditioning (see table). In the first experiment bees had to distiguish between two mixtures of odours (AB+ CD-), in a second experiment they were confronted with four single odours (A+B+ C-D-). In a third experiment they received a simple two odour conditioning (A+ B-). All odours were balanced. Animals received five learning trials and got an unilateral TTX-injection (1 µm, 0. 5 nl) 150 min after training into the mushroom body (α-lobe). 50 minutes after injection bees were tested for their response to the learned odours.

TTX-injection into the MB showed no influence on the PER elicited by the US (sucrose solution) applied to the antennae. Ringer-injected bees (as above) were used as control groups.

	Conditioning (5 trials)	Injection	Retention
Group 1	AB+ CD-	TTX	No
	AB+ CD-	Ringer	Yes
Group 2	A+ B+ C- D-	TTX	No
	A+ B+ C- D-	Ringer	Yes
Group3	A+ B-	TTX	Yes
	A+ B-	Ringer	Yes

The bees learned the tasks and showed no differences during acquisition between treatment groups. The performance of the 6 groups during the extinction test differed according to the kind of injection and the kind of the previous learned conditioning. All control groups showed normal retention. After TTX-injection Group 1 and 2 could not distiguish the previously reinforced (CS+) from the previously non-reinforced odours (CS-), whilst the animals of group 3 were still able to retrieve information they learned about the CS+ (odour A). Honeybees seem to need both α-lobes for the retrieval of learned olfactory information at a discriminative task. Therefore the MB seems to be necessary for retrieving olfactory memory in the honeybee. In a very simple conditioning (group 3) other neuropils or only one MB seem to be sufficient for memory retrieval. Supported by the DFG (SFB 515/C5), DAAD / MAE PROCOPE grant Nr. 9910368 / 00352UE

Cellular mechanisms of odor learning in honeybees: Combining electrophysiology and Ca^{2+} imaging

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Detection of the temporal coincidence of the conditioned stimulus (CS) and unconditioned stimulus (US) is an essential feature of neurons involved in classical conditioning. The mushroom bodies of the insect brain are important structures for olfactory learning and memory and its intrinsic Kenyon cells are candidate neurons for coincidence detections. In the honeybee they receive olfactory information (CS) from cholinergic projection neurons from the antennal lobe and reward information (US) from octopaminergic VUM neurons of the suboesophageal ganglion. We are investigating possible mechanisms how Kenyon cells detect the coincident activation of cholinergic and octopaminergic pathways using patch clamp and imaging techniques in-vitro.

Cultured honeybee Kenyon cells express neuronal nicotinic acetylcholine receptors (Goldberg et al., 1999; Déglise et al., 2002). The receptor is a cation-selective channel with a high Ca^{2+}-permeability. Thus, activation of the CS pathway induces membrane depolarization and Ca^{2+} influx in Kenyon cells.

For Ca^{2+}-imaging experiments cultured Kenyon cells were loaded with FURA-2-AM or FLUO-4-AM (6.5 μm) for 30 minutes and imaged with a cooled CCD camera (sample

rate 10 Hz). Pressure applications of acetylcholine (ACh, concentration 200 µm, pulse duration 100-200 ms) induced fast and long lasting Ca^{2+} signals. The Ca^{2+} signal comprises of Ca^{2+} influx through the nicotinic receptor and through voltage-sensitive Ca^{2+} channels. Blocking of voltage-sensitive sodium and potassium channels with TTX and TEA reduced the Ca^{2+} signal by about 80%. This indicates that most of the Ca^{2+} signal comes through voltage-sensitive Ca^{2+} channels.

Kenyon cells also express an octopamine receptor which is coupled to the cAMP/PKA pathway and may induce an intracellular Ca^{2+} signal. But until now the cellular consequences of the octopamine receptor activation are not identified. We are currently investigating possible interactions between the nicotinic ACh and the octopamine receptor.

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640 Optical imaging of Kenyon cell activity in the mushroom body during odor perception and odor learning in the honey bee, *Apis mellifera*

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We studied how olfactory information is processed during odor perception and odor learning in the higher-order integration center of the insect brain - the mushroom bodies (MB). The MB receives olfactory input from the first-order olfactory neuropil, the antennal lobe, via projection neurons, visual input from the visual ganglia via the anterior-superior optic tract, and mechanosensory input via unknown pathways. These higher-order sensory inputs are segregated in the calyces of the MB (olfactory: lip region; visual: collar; mechanosensory: predominantly basal ring). The MBs are known to be involved in neural plasticity underlying associative olfactory learning. Since the neuron representing the reward function in appetitive olfactory conditioning, the VUMmx1, converges with the olfactory pathway in the lip region of the MBs it has been concluded that the synapses between projection neurons and intrinsic MB neurons, the Kenyon cells, provide a substrate for learning-related plasticity. It is not known how olfactory stimuli are represented in the lip region by the Kenyon cells, and it is unknown whether and how this representation might change with olfactory learning.

In an attempt to address these questions using Ca^{2+} imaging we asked whether Kenyon cells of the lip region of the MB compute olfactory information in spatially ordered functional subunits as is the case in the antennal lobes, or whether odor information is processed in the form of a spatially distributed ensemble code and how this olfactory code may be changed during odor-learning.

In order to monitor Kenyon cell activity, we retrogradely labeled Kenyon cells with a dextran coupled Ca^{2+}-sensitive dye, which was injected into the ventral part of one output region of the MB, the α lobe. The somata and dendrites of the selectively stained Kenyon cells were then optically recorded *in-vivo* in the lip-neuropil of the calyces. Single odors activated 2 - 4 % of the measured cells which appeared distributed over the entire lip of the calyces and showed no clustering. Half of the responding cells were

tuned to and reliably activated by some odors, whereas the other half responded variably. A third of the cells were activated by more than one of the four tested oders.

To investigate learning effects bees were differentially conditioned during the imaging experiments. Olfactory conditioning changed the response-strength and the -dynamics of individual cells. We observed [Ca^{2+}]-increase or -decrease in response to the rewarded as well as to the unrewarded odor after training.

Our results suggest that the stable and odor-specific code of the presynaptic antennal lobe-projection neurons is transformed into a complex Kenyon cell-activity-pattern which represent both: odor-identity in form of stable odor specific Kenyon cell ensembles and information about odor-experience in form of variably and dynamic responding Kenyon cells.

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Dendritic localization of the Arg3. 1/Arc mRNA binding protein Zinki is negatively regulated by synaptic activity 641

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While Arg3.1/Arc has a key role in synapse specific modifications, nothing is known about the mechanisms that govern Arg3.1/Arc mRNA transport into dendrites. To address this issue, we developed the Tri-Hybrid method for the *in vivo* reconstruction of specific RNA-protein interactions. Using this technique in a genetic screen we identified several clones that specifically interact with Arg3.1/Arc mRNA but not with perikaryal-control RNAs. One of these proteins we have named Zinki because it contains a domain of repeated zinc fingers required for the specific binding to Arg3.1/Arc mRNA. The expression of Zinki is predominantly dendritic. *In vitro* and *in vivo* experiments indicate that Zinki co-localizes with and binds to GRF1, a GDP/GTP exchange factor for small GTPases like ras and rac. Moreover, we find that upon dendritic lamina specific stimulation, Zinki vacates those dendritic regions in which translation of the Arg3.1/Arc mRNA is enhanced suggesting that Zinki may control translation of the Arg3.1/Arc mRNA in this compartment.

Arg3. 1 is associated with the NMDA-receptor complex and is required for memory formation 642

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Enduring forms of synaptic plasticity like long-term memory and long-term potentiation (LTP) require alterations in the molecular composition and structure of neurons and are dependent on RNA and protein synthesis. Functional plasticity might therefore be achieved by activity-dependent changes in the expression of specific genes. The expression of Arg3.1/Arc is robustly induced by LTP stimulation. Moreover, following induction Arg3.1/Arc mRNA and protein are rapidly distributed throughout the dendritic

arbor and can be specifically targeted to stimulated synapses. While the expression of Arg3.1/Arc is unique among all known genes and establishes a strong link to synaptic plasticity, the precise functional role of Arg3.1/Arc has so far been elusive. Biochemical analysis demonstrates that Arg3.1/Arc is associated with the NMDA (N-methyl-D-aspartate) receptor complex. This association is not seen in animals that lack PSD95/SAP90, a protein that can directly bind to the NMDA-receptor. Furthermore, Arg3.1/Arc is present in the postsynaptic density (PSD). The PSD provides a highly organized biochemical machinery which, among others, anchors signaling molecules to ion channels and therefore coordinates activity-dependent changes on the postsynaptic side. We have generated Arg3.1/Arc knockout animals, in which we deleted the complete gene. These animals exhibit specific deficits in the acquisition of hippocampus-dependent learning tasks. Namely, Arg3.1/Arc mutants show a dramatically weaker learning performance in the Morris water maze place navigation. Furthermore, during classical fear conditioning both cue and context dependent long term memory consolidation is impaired in Arg3.1/Arc mutant mice. Thus, Arg3.1/Arc may act to maintain the structural or functional integrity of the NMDA-receptor complex, and thereby influence the signaling pathways that control the magnitude and specificity of synaptic plasticity leading to structural changes underlying learning and memory.

643 Extinction and re-consolidation in the honeybee *Apis mellifera*: Two interfering processes?

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Protein synthesis inhibitors cause amnesia in a variety of learning paradigms when applied around the time of training in several species. Hence, consolidation of a transient, unstable short-term memory into a stable and long-lasting long-term memory requires synthesis of new proteins (Davis and Squire, 1984).

In addition, the activation of consolidated long-term memory by reminder-cues induces a re-consolidation process that is again susceptible to protein synthesis inhibitors (Nader *et al.*, 2000). Conflicting with this re-consolidation of activated memories are studies showing that the presentation of a reminder-cue without reinforcement initiates extinction of responses. Extinction is thought to require the aquisition of new information like the original learning process and has also been shown to be dependent on the synthesis of new proteins (Vianna *et al.*, 2001). In line with these conflicting studies it has been demonstrated in rats, that inhibition of protein synthesis after retrieval leads either to an inhibition of re-consolidation or to the inhibition of extinction. This might be due to different tasks or brain regions treated with the inhibitors (Berman and Dudai, 2001, Nader *et al.*, 2000).

In this study we aim to contribute to a better understanding of the relation of re-consolidation and extinction. We are examining re-consolidation and extinction in the honeybee *(Apis mellifera)* using an appetitive olfactory conditioning paradigm, the PER (proboscis extension response) conditioning. A training period is followed 24 h later by an single or multiple exposure to the conditioned stimulus alone (extinction/reminder

stimulation). Our data indicate a highly sensitive balance between extinction learning and reminder effect as indicated by sometimes stronger and sometimes less strong reduction of retention scores. The extinction effect becomes enhanced with multiple extinction trials.The effect of protein synthesis inhibition following an extinction trial supports this conclusion: in some experiments a re-consolidation effect is apparent as indicated by lower retention scores as compared to the control group. We therefore conclude that memory formation after an exposure to the learned stimulus might induce both memory reducing and memory enhancing effects on the original memory trace.

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A modified version of the unique cue theory accounts for olfactory compound processing in honeybees

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We investigated the capability of honeybees to discriminate between single odorants, binary olfactory mixtures and ternary olfactory mixtures in olfactory PER-conditioning. In Experiment 1, three single odorants (A+, B+, and C+) and three binary mixtures of these odors (AB+, AC+, and BC+) were reinforced whilst the ternary compound, consisting of all three odors (ABC-), was non-reinforced. In Experiment 2, only one single odorant (A+) and one binary olfactory compound (BC+) were reinforced whilst the ternary compound (ABC-) consisting of the single odor and the binary compound was non-reinforced. We studied whether bees can solve these problems and whether the course of differentiation can be predicted by the Unique Cue Theory, a Modified Unique Cue Theory or Pearce´s Configural Theory. Honeybees were not able to differentiate reinforced from non-reinforced stimuli in Experiment 1. However, summation to ABC at the beginning of training contradicts the predictions of Pearce´s Configural Theory. In Experiment 2, differentiation between the single odorant A and the ternary compound developed more easily than between the binary compound BC and ABC. This pattern of differentiation is in line with a Modified Unique Cue Theory and Pearce´s Configural Theory. However, summation to ABC at the beginning of training again was at odds with Pearce´s Configural Theory. Thus olfactory compound processing in honeybees can best be explained by a Modified Unique Cue Theory.

645 Sucrose responsiveness and behaviour in honey bees and fruit flies

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In the honey bee, individual sucrose responsiveness can be measured easily using the proboscis extension response. Sucrose responsiveness correlates with different forms of learning and foraging behaviour and is thus an excellent indicator of the behavioural state of a bee.

We tested whether individual sucrose responsiveness can also be used as a behavioural indicator in different genetic variants of *Drosophila*. When the tarsus of an individually mounted fly is stimulated with a sucrose concentration above its response threshold, the fly extends its proboscis. Sucrose responsiveness was determined by measuring the occurrence of proboscis extension at stimulation with a series of sucrose concentrations.

We compared the sucrose responsiveness of the two naturally occurring *Drosophila* variants for^{s}("sitters") and for^{R} ("rovers"). The two variants differ in their larval foraging behaviour and in their levels of cGMP-dependent protein kinase (PKG). Sitter larvae have shorter foraging trails and lower PKG levels than rover larvae. To test whether observed differences in sucrose responsiveness were a result of differences in PKG levels, we also analysed the strain for^{s2}, which is a sitter mutant strain made on a rover genetic background.

We tested male and female flies of the variants sitter, rover and for^{s2} at 1, 2 and 3 weeks old and found that rovers were always more responsive to sucrose than sitters and for^{s2} flies. These results show that sucrose responsiveness in *Drosophila* correlates with PKG levels. Similar to the differences in foraging trails, the differences in sucrose responsiveness between sitters and rovers were greater after 2 hours of starvation than after 24 hours of starvation. We also tested habituation of proboscis extension in adult sitters, rovers and for^{s2} flies. In contrast to the honey bee, habituation was independent of individual sucrose responsiveness in all strains. Interestingly, rovers showed less habituation than sitters and for^{s2} flies, which demonstrates an additional effect of PKG levels on habituation in fruit flies.

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646 The Role of the Mitogen-Activated Protein Kinases in Learning

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Mitogen-activated protein kinases (MAPKs) are components of cascades mediating growth and differentiation but also stress in mammalian cells. The cascades are orga-

nized hierarchically into three modules that are activated by distinct stimuli and signal inputs. External signals activate the MAPKs via hierarchically-organized kinases that activate each other. A module that mediates inputs from growth factors, cytokines and stress factors such as ultraviolet radiation and heat shock is the Ras/Raf/MEK/ERK cascade. In this particular cascade, the MAPK (ERK) is phosphorylated by the MAPK-kinase (MAPKK) MEK, which in turn is activated by the MAPKK-kinase (MAPKKK) Raf. The MAPKKKs receive their inputs from GTPases or other protein kinases that are connected to G-protein-coupled receptors or cell surface receptors. Since protein kinases like the cAMP-dependent kinase (PKA) and protein kinase C (PKC), which play central roles in learning and memory formation, can interact with the MAPK cascade we are interested in a possible function of the MAPKs in learning and memory formation.

In rats and mice many studies demonstrate that the MEK cascade plays a crucial role in differentiation, growth, apoptosis, development and stress. However, only a few investigations point to a function of MEK in neuronal plasticity and learning. So it has been demonstrated that activation of the MAPKK, MEK is required for induction of long-term potentiation (LTP) in the hippocampus. Studies in *Aplysia* also show that MEK is important for long-term facilitation but not necessary for short-term facilitation. In rats, the MEK activity is required for formation of long-term memory (LTM) in fear conditioning

The goal of this work is to investigate the role of the MAPK-cascade in honeybee associative learning and hence in LTM formation. In a first step we tested whether mammalian antibodies against different members of the MEK family can be used to identify the corresponding members in the honeybee brain. Using a combination of western blotting and pharmacological tools we demonstrate that we can apply these antibodies to investigate the localization and the function of the MAPKs cascade in honeybees. Immunohistochemical studies show that different members of the MAPK cascades are concentrated in the mushroom bodies and the antennal lobes of the honeybee. These brain areas are important for associative olfactory learning. In a behavioral assay we tested whether blocking of MEK interferes with learning and memory formation. Our results show that inhibition of MEK activity during learning impairs long-term memory formation but does not affect learning short- and mid-term memory. These findings suggest that MEK, localized in antennal lobes and mushroom bodies, may play a crucial role in LTM formation.

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Zenk immunoreactivity after reversal learning in the avian forebrain

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The expression of immediate-early genes (IEGs), including ZENK, is induced in many vertebrate species in response to sensory stimulation and during behavioural activity. It has also been linked to learning and memory formation. In birds, IEG expression has

been extensively used to map brain areas related to sexual behavior and vocal learning (1, 2) but rarely to investigate the contribution of different brain areas in cognitive learning tasks. We examined the behavior-driven ZENK protein expression in the forebrain of pigeons during a serial color reversal task, which involves more complex learning and behavioral strategies than a simple color discrimination. Pigeons *(Columba livia)* were tested either in a previously acquired color discrimination (red versus green) or in a color reversal paradigm, in which stimulus-reward contingencies were reversed as soon as 15 successive correct responses were obtained. With each new reversal, responses to the previously rewarded stimulus (S+) were punished, whereas responses to the previously incorrect stimulus (former S-) were rewarded. At the end of the sessions the brains of the subjects were subjected to standard immunocytochemistry using polyclonal antibody against Egr-1 (Santa Cruz Biotechnology). Immunoreactive (ir) cell nuclei in different brain areas were automatically counted (Soft Imaging System) and visually controlled. Analysis of the primary and secondary visual processing areas of the thalamofugal (geniculo-cortical) and tectofugal (extrageniculo-cortical) pathways revealed no difference in the expression pattern of Egr1-ir cells between the non-reversal group (n=4) and reversal group (n=5). Induction of the ZENK protein in general was higher in thalamofugal areas (HIS, HD, HA) than in the tectofugal pathway (Ep, E). In contrast, the number of ZENK-ir cells was significantly higher in the neostriatum frontolaterale (NFL) and lobus parolfactorius (LPO) of the reversal than in the non-reversal group. Lesion data indicate that both visual pathways are necessary in reversal learning (3-6). These pathways project onto NFL, where information is combined (4, 7). The LPO participates in the control of motor behavior (avian basal ganglia) and is essential for behavioral flexibility needed in serial reversal. Thus, our preliminary results indicate that reversal learning as compared to a simple color discrimination induces an increase in the ZENK protein expression in association and motor output areas, where information from the different visual pathways is integrated.

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648 NMDA receptors in the pigeon prefrontal cortex - a role for working memory?

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The avian Neostriatum caudolaterale (NCL) is considered the functional equivalent to the prefrontal cortex (PFC) in mammals, based on neuroanatomical, electrophysiological and behavioral evidence. Lesions of the NCL were found to cause deficits in working memory tasks (1), as well as in other tasks probing prefrontal function (2). NMDA receptor blockade in the NCL leads to deficits in a color reversal task (3). In this study we investigated the role of NMDA receptors in the pigeon NCL for working memory. We tested the animals? performance in two tasks with different recruitment of working

memory: simultaneous matching to sample (SMTS) and delayed matching to sample (DMTS), after local injection of the competitive NMDA antagonist DL-AP5 into the NCL and compared it to performance of the same subjects after a saline injection. The results show a significant increase in errors under DL-AP5 - not only in the DMTS task, requiring working memory, but also in the SMTS task, which requires no working memory. We conclude that the relevant function of NMDA receptors in the avian PFC seems to be selection of the contextual adequate response rather than keeping information on-line in working memory.

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Performance in a four-arm baited eight-arm radial-maze after microinjections of glutamate antagonists in the nucleus accumbens 649

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The nucleus accumbens (NAC) is considered to be an important neural interface between corticolimbic and motor systems of the brain. Glutamatergic neurons from multiple limbic sites project to the NAC which relays these signals to subpallidal motor effector sites. Thus the NAC seems to be strategically located to translate e.g. hippocampal signals into action. Therefore, it is not surprising that inhibition of glutamatergic synapses alters behavior in spatial memory tasks in a similar way to those changes seen after lesions of the hippocampus. However, most studies demonstrated a special role of NMDA receptors in acquisition, but only few data exist about the involvement of different glutamate receptors in performance of these tasks. Therefore, we investigated the effect of microinjection of the nonselective glutamate antagonist kynurenic acid (KA), the NMDA antagonist 2-amino-5-phosphonopentanoic acid (AP-5) and the non-NMDA antagonist 6,7-Dinitroquinoxaline-2,3-dione (DNQX) in the NAC of adult male wistar rats on performance in a four-arm baited four-arm unbaited test in an elevated open eight-arm radial-maze.

For each rat four arms were randomly chosen to be rewarded and the following behavioral parameters were determined: total time to complete a trial (time), working memory errors (WME) and reference memory errors (RME). Initially, rats were trained until they reached baseline performance. After this initial training period all rats were stereotaxically implanted with bilateral guide cannulas aiming at the NAC under chloralhydrate anesthesia. After three days of recovery, training continued until rats reached baseline performance again. First, the effect of 4.5µg/0.3 µl KA in 0.3µl PBS or vehicle was tested 10 min after microinjection. Thereafter the effect of 1µg AP-5 and 0.75µg DNQX in 0.3µl PBS and vehicle was similarly tested in randomised order. After each microinjection the rats were trained for one day without injection. Since all rats showed baseline performance on these days, microinjections were considered not to interfere with each other. After the experiments the brains of all rats were histologically processed for Nissl staining in order to check the correct placement of the injection sites.

Microinjection of KA induced impaired performance in time, WME and RME ($p<0.05$). AP-5 had no effect on these parameters while DNQX increased RME ($p<0.05$).

These results indicate that glutamate receptors in the NAC play an important role in the performance on a spatial working/reference memory test. Non-NMDA receptors seem to be especially involved in these processes.

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650 Behavioural effects of neonatal excitotoxic lesions of the rat entorhinal cortex

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The entorhinal cortex possesses direct efferent projections to the hippocampal formation via the perforant path and influences a variety of limbic-striatal systems. Thus, the entorhinal cortex is in a strategic position to mediate cognitive functions in mammals. A developmental anomaly of the entorhinal cortex is reported in schizophrenic patients that is probably relevant for aspects of the symptoms.

The present experiments investigated the effects of bilateral neonatal (postnatal day 7; PD7) ibotenic acid (1,3µg/0,2µl) lesions of the entorhinal cortex (n=6) on motivation, locomotor activity and sensorimotor gating in adult male wistar rats. Sham lesioned (n=8) and naïve rats (n=8) served as controls. The behavioural tests were started at PD100. Locomotor activity was measured in prepubertal (PD35), young adult (PD56) and adult (PD110) rats. Adult rats were trained to press a lever for food reinforcement according to a continuously reinforced schedule for four days. Thereafter, rats were subjected to one experimental session during which operant responding was rewarded according to a progressive ratio schedule with the highest ratio obtained before the animal ceases responding termed breakpoint. The number of pellets delivery was changed every second minute. Each experimental session lasted 30 min. Spontaneous locomotor activity was assessed for 35 min in a photocell activity box. Additionally, the effect of neonatal lesions on prepulse inhibition (PPI) of the acoustic startle reflex, an operational measure of sensorimotor information processing, was analyzed. Finally, all animals were perfused intracardially with 4% paraformaldehyd. Then, the location and extent of the lesions were determined histologically in Nissl-stained sections.

Neonatal injections of ibotenate produced thinning of tissue in the rat entorhinal cortex. Rats with lesions of the entorhinal cortex showed a leftward-shift of breakpoints and decreased cumulative lever presses in a progressive ratio schedule of operant behaviour. Locomotor activity was not altered by neonatal lesions during all developmental stages but was increased in adults as compared to prepubertal and young adult rats. PPI was not altered in neonatally lesioned rats.

The results presented here indicate that neonatal lesions of the entorhinal cortex reduced the motivational state of rats to lever press for food pellets, as measured by the progressive ratio schedule of operant behaviour. This might indicate that the aversive value of the non-reward is greater in lesioned rats than in sham-lesioned and naïve control rats.

No motoric impairment could be observed. Thus, reduced lever pressing is not due to diminished locomotor activity. The reduced motivation to lever press in rats may reflect a state of avolition which is one of the negative symptoms of schizophrenia in humans. Additionally, there was no PPI deficit in ibotenate-lesioned rats. Thus, neonatal lesions of the entorhinal cortex do not disturb sensorimotor information processing.

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Effects of neonatal lesions of the rat medial prefrontal cortex on adult behavior 651

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According to the Two-Hit-Model of schizophrenia, brain damage at early stages of ontogeny (first hit) leads to a higher vulnerability of the brain to adverse events later in life (second hit). Pubertal stress is considered to be a major precipitating factor in the onset of psychosis. In the present behavioral pharmacological study we addressed the question whether neonatal excitotoxic lesions of the medial prefrontal cortex (mPFC) in rats lead to altered behavior and to enhanced vulnerability of the brain to drug effects during and/or after puberty or if the lesion effects are compensated. Results of some tests were compared to effects of adult lesions in the mPFC.

Neonatal (postnatal day 7) and adult neuronal lesions were induced by bilateral microinjection of ibotenate (0,2 µg in 0,3 µl PBS) into the mPFC in anesthetized male Wistar rats, sham-lesioned rats were microinjected with the same amount of the vehicle. Additionally, neonatal operated rats were injected with 20 mg/kg dexamethasone once at postnatal day 49 in order to mimic a stress attack. Behavioral tests were started 9 weeks after ibotenate-injection in both neonatal and adult lesioned rats. Working memory was tested in a spatial continuous delayed alternation task, using a T-maze. Errors were divided into two types, a `first error` (type 1) was made when the rat had entered the arm, which was the correct one in the previous trial and a `perseverative error` (type 2) was made when the rat continued to choose the incorrect arm. Locomotor activity was tested for 35 min in an open field with and without 0.5 mg/kg apomorphine. After the experiments the brains of all neonatal lesioned rats were histologically processed for Nissl staining in order to determine the extent of the lesions.

Bilateral infusion of ibotenic acid into the mPFC on postnatal day 7 leads to visible scars and thinning of the mPFC as well as to ventricular enlargement in adult rats. Early postnatal lesions of the mPFC had no effect on type 1 errors, whilst type 2 errors were significantly enhanced compared to sham-lesioned rats ($p < 0.05$). Additional challenge with dexamethasone on day 49 had no effect. Spontaneous locomotor activity was unaltered by neonatal lesions or dexamethasone challenge. Apomorphine increased locomotor activty in non-challenged but not in dexamethasone-challenged rats ($p < 0.05$) irrespective of whether or not the animals were lesioned.

Our data indicate that early damage of the mPFC spared working memory function but lead to a perseveration like deficit in a T-maze alternation task. Apomorphine induced hyperactivity was reduced in dexamethasone-treated rats.

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652 The *white* ABC transporter of *Drosophila* is needed for high-temperature reinforcement processing in the heat-box learning paradigm.

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The ABC transporters constitute a large class of proteins that transfer substances across a membrane in an ATP-dependent fashion. Indeed, mutated ABC transporters can cause human diseases like cystic fibrosis and Tangiers disease. In *Drosophila*, the *white* mutation was isolated nearly 100 years ago and subsequently identified as coding for an ABC transporter. Little outside its role in pigmentation of the retina has been determined.

In this study, *white* mutant flies were found to be abnormal in a spatial active avoidance learning paradigm that uses high-temperature as reinforcement. In this so called heat-box paradigm, memory is expressed by the continued avoidance of a chamber-half associated with a high temperature. *white* mutant flies show a defect during training and very little measurable memory, as do *brown* and *scarlet* mutants, which are known to be White heterodimer binding partners. The *white* defect can be rescued with a chromosomal translocation including a wild-type *white* gene. In a simpler thermosensitivity task, *white*-mutant flies can avoid those temperatures used in the learning experiments, although not as well as wild-type flies. In a parametric analysis of training duration and reinforcer intensity, *white*-mutant flies show a lower asymptotic memory score than wild-type flies with prolonged training. Furthermore, higher reinforcement intensity led to memory scores approaching normal in the *white*-mutant flies. Interestingly, such a pattern of results is predicted by current models of associative learning for treatments that compromise reinforcement processing. It is, therefore, suggested that the *white* defect is in reinforcement processing.

653 Blood pressure responses in the fear-conditioned mouse

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Fear conditioning studies in mice and rats generally address the level of conditioned fear on the basis of behavioral observations such as freezing. The feasibility of behavioral assessment of the conditioned fear response under stress-free conditions in the home cage is limited due to low exploratory behavior of mice in their habituated environment.

We have therefore previously analyzed the heart rate (HR) responses of freely moving mice by telemetry to assess the level of conditioned tone-dependent fear. HR was demonstrated to be a useful indicator for attention and emotional memory when monitored under otherwise stress-free conditions (Stiedl & Spiess, 1997, Behav. Neurosci. 111:703-711; Stiedl et al., 1999, Behav. Brain Res. 104:1-12).

We have now established telemetric measurements of mean arterial pressure (MAP) in conscious male C57BL/6N mice via the abdominal aorta. In the auditory fear conditioning paradigm, we assessed MAP in the home cage during the tone-dependent memory test. Expression of emotional memory was tested 24 hr after training with either single or multiple tone-shock pairings. After the onset of the aversively conditioned tone stimulus, MAP increased from baseline values of approximately 90 mmHg that were in the upper range of circadian light-phase MAP to values of approximately 130 mmHg exceeding the maximum MAP of approximately 105 mmHg observed during the circadian cycle. Furthermore, differences in the temporal dynamics of HR and MAP responses became apparent. HR exhibited an almost instantaneous acceleration to maximum values in the first 30 s after tone onset, whereas MAP increased steadily to peak values 90 s after tone onset and showed a delayed recovery to baseline. The sluggish MAP increase during tone exposure was absent in non-conditioned mice. An initial very fast peak of MAP at tone onset was observed in approximately 50 % of tested mice, which is interpreted to reflect an attention response rather than an expression of conditioned fear.

Previous studies in rats (Iwata & LeDoux, 1988, Behav. Neurosci. 102:66-76) concluded that only MAP but not HR would serve as indicator of conditioned fear, and the MAP responses indicative of emotional memory were expressed in the initial 10 s of tone presentation. In contrast, the results of the present study demonstrate that both MAP and HR were both indicative of conditioned auditory fear in mice. However, the time course of HR and MAP readjustment was markedly different.

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Transcranial direct current stimulation (tDCS) of the primary motor cortex enhances implicit motor learning **654**

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Transcranially applied weak direct currents are capable of modulating motor cortical excitability in the human. Anodal stimulation enhances excitability, cathodal stimulation diminishes it. Cortical excitability changes accompany motor learning. Thus, the aim of this study was to test if an external excitability modulation of cortices known to be involved in motor learning could modify these processes. In primary motor, premotor and prefrontal cortices anodal, cathodal or placebo tDCS were applied during performance of an implicit sequential finger movement task (serial reaction time task, SRTT) contralaterally to the performing hand. Anodal tDCS of the primary motor cortex diminished reaction times during performance of the SRTT, while cathodal stimulation of the same area and tDCS of the remaining cortices had no effect. Error rates and variabilities were identical in all conditions. We conclude that the primary motor cortex is involved in the acquisition and early consolidation phase of implicit motor learning.

655 Increased mortality and spatial memory deficits in TNF-α deficient mice after experimental pneumococcal meningitis

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Objectives: Tumor necrosis factor α (TNF) is critically involved in inflammation and cellular immune response during bacterial infections. TNF may participate in hippocampal neuronal injury in meningitis. In a mouse model of pneumococcal meningitis, the influence of TNF-deficiency on mortality and on spatial memory performance was investigated.

Methods: 57 TNF-deficient mice (males, 2-3 months) and 55 sex- and age-matched controls (C57Bl6) were trained to find a hidden under-water platform within less than 90 s (18 trials over 3 days). Swim tracks and the latency to escape from the water were recorded by a video camera. Thereafter, mice were infected by injection of 4 log CFU Streptococcus pneumoniae into the right forebrain. 30h later therapy was initiated with ceftriaxone (100 mg/kg twice daily for 5 days). Motor performance was measured by the tight rope test. Beginning 7 days after infection, water maze was performed (daily in the first week, then three times per week), and mice were killed 6 weeks after infection.

Results: During swim training in the first 3 days no differences were seen in the water maze between TNF-deficient mice and controls. After infection, tight rope test showed severe impairment during the acute phase of meningitis. 35 (61%) TNF-deficient mice and 22 (40%) controls died within 6 days (Fisher's exact test: $p=0.04$). All other animals fully recovered from the infection. TNF-deficient mice surviving pneumococcal meningitis took substantially longer to reach the hidden platform than controls ($p=0.02$) and the distance of swim tracks was significantly longer ($p=0.02$). The swim speed in both groups was similar ($p=0.59$).

Conclusions: In pneumococcal meningitis TNF deficiency caused increased mortality and deficits in spatial memory, i.e. the increased vulnerability of TNF-deficient animals to infection outweighs possible detrimental effects of TNF release within the CNS.

656 Mice deficient for the extracellular matrix glycoprotein tenascin-R show increased hippocampal polyspiking activity and shifted thresholds for induction of long-term potentiation and depression

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The extracellular matrix glycoprotein tenascin-R (TN-R) is highly expressed in the pyramidal layer of the CA1 region of the hippocampus, being one of the major compo-

nents of perineuronal nets surrounding parvalbumin-positive interneurons. Mice deficient in TN-R exhibit reduced perisomatic inhibition and saturated levels of excitatory synaptic transmission, similar to that found in rats after experimentally induced epilepsy. As *in vitro* correlate of epileptic activity, we analyzed the ability of hippocampal slices to develop multiple population spikes in response to repetitive stimulation of Schaffer collaterals. The area and numbers of secondary spikes turned out to be significantly higher in TN-R mutants as compared to wild type controls. Previously we reported that long-term potentiation (LTP) induced by the θ-burst stimulation of Schaffer collaterals in the CA1 region is impaired in TN-R deficient mice. Here, we report that pairing of low-frequency presynaptic stimulation with a strong depolarization of postsynaptic CA1 pyramidal cells to 0 mV induced normal LTP of approximately 190% in TN-R mutants. However, pairing of presynaptic stimulation with a weaker depolarization to -10 mV produced LTP of approximately 150% in wild-type mice, but not in TN-R deficient mice. Pairing at -20 mV produced long-term depression (LTD) in TN-R mutants, not affecting synaptic transmission in wild-type mice. Interestingly, the increased threshold for induction of LTP correlated with an elevated number of perforated asymmetric axospinous synapses in the stratum radiatum of TN-R deficient mice, whereas the density of non-perforated synapses was not changed compared to wild-type mice. These observations show that reduced levels of perisomatic inhibition in TN-R deficient mice are detectable at the population level as increased polyspiking activity. The impaired inhibition likely promotes metaplastic changes in the CA1 region that appear as a shift in the threshold of induction of long-term modifications and elevated numbers of perforated synapses.

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Constitutive proteolytic activity is required for short-term plasticity of cultured *Aplysia* sensorimotor synapses **657**

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The mechanisms underlying short term facilitation in Aplysia sensory-motor synapse can be subdivided into two processes: (a) a spike duration dependent process (sensitization) that results from 5HT induced PKA activation and (b), a spike duration independent process (dishabituation) that results from PKC activation. As a result of PKA activation the potassium conductance is reduced, leading to spike broadening and enhanced calcium influx. The cellular mechanisms underlying PKC dependent synaptic dishabituation are not entirely understood. It was suggested that vesicles mobilization, alterations in the release mechanism or local activation of specialized calcium channels might be involved.

In contrast to earlier reports, we found that calpains (calcium activated cystein neutral proteinases), are involved in the cascade of events leading to synaptic habituation and dishabituation.

Application of the membrane permeable calpain inhibitors calpeptin or the nonspecific proteasome inhibitor MG132 increases the rate of synaptic habituation and inhibits 5HT-induced synaptic dishabituation. On the other hand, sensitization is not affected by the inhibitors.

The results are consistent with the hypothesis that inhibition of constitutive proteolytic activity slow down or prevent the translocation of synaptic vesicles to the release sites. As a consequence the rate of synaptic habituation is accelerated in the presence of the inhibitors, and after massive habituation, 5HT application does not lead to dishabituation. These results demonstrate that constitutive proteolytic activity is necessary for the induction of short-term neuronal plasticity.

658 Endogenous IL-6 is involved in hippocampal long-term potentiation and spatial learning

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Interleukin-6 (IL-6), a cytokine and member of the neuropoietin family, is not only produced by immune cells but also by glial cells and certain types of neurones in the brain. Pharmacological studies demonstrated pleiotropic actions of IL-6 on a number of neuro-endocrine functions. However, it is still unclear, whether activation of a discrete neuronal population triggers IL-6 production under non-pathological conditions and whether this has functional implications.

We found *in vivo* and *in vitro* that IL-6 gene expression is substantially increased in the dentate gyrus during long-term potentiation (LTP), a process considered to underlie certain forms of learning and memory. The increase in gene expression was long lasting, specific to potentiation and could be prevented by application of the NMDA receptor antagonist, (±)-2-amino-5-phosphonopentanoic acid (AP-5). Blockade of the function of endogenous IL-6 by a neutralizing antibody (IL-6AB), applied i.c.v. 90 minutes after tetanization, caused a prolongation of LTP in freely-moving animals for at least 4 hours. Since no comparable effects were observed when the antibody was administered before or immediately after LTP induction, IL-6 appears to have a specific function in LTP maintenance. To test whether IL-6 is also involved in certain types of learning we employed the Y-maze spatial alternation task, where animals have to learn a footshock-motivated left-right spatial alternation reaction as earlier described (*Neuroreport 5, 2061-2064, 1994*). 90 min after the end of the training session (40 trials; 1 min inter-trial interval), the same amount of IL-6AB as used for LTP *in vivo* was injected i.c.v.. Control rats received the same volume of pre-immune serum. When retention was tested 24 hours after training by the same behavioral procedure (relearning), IL-6AB treated animals showed significantly less errors as compared with the control group. No group differences in other behavioral parameters such as spontaneous activity; number of fecal boli; inter-trial crossings; footshock sensitivity could be detected during retention.

Collectively, our data suggest that an increased production of IL-6 during LTP and learning may serve to control decisive mechanisms in the consolidation of LTP and memory.

Effects of MK-801 on learning of instrumental food-acquisition behavior in rats and its neuronal base. 659

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The glutamatergic NMDA-receptor is an important key-player required for many types of learning and memory formation. Various experiments *in vitro* and *in vivo* have shown that NMDA-receptor blockers can suppress a form of long-term potentiation, as well as distinct forms of learning. It is known that systemic administration of the non-competitive NMDA-receptor antagonist MK-801 influences acquisition of new behaviors, particulary instrumental behavior. Currently, we develop a method which will allow us to train rats in an instrumental cage and simultaneously to record single unit activity in the cingulate anterior cortex and the hippocampus in freely moving rats. Long Evans rats are trained to press pedals to receive portions of foods in distinct feeders. The training consists of different stages: first, rats are introduced to the feeders rewarded with small food pellets; second, the rats have to learn to turn around and to move in the direction of pedal location; third, they should approach the pedal to obtain another reward as a reinforcement; and fourth, to press the pedals. MK-801 will be injected i.p. (0.1 microg/kg) 30 min before training the fourth stage, i.e. to press the pedals. Interestingly, in some rats we observed a significant quicker acquisition time of new behavior and the animals learned even under the influence of the NMDA-receptor blocker. One possibility to explain this preliminary finding is that MK-801 administration led to a different neuronal organization of the newly formed behavior. To prove this hypothesis we record neuronal activity in the hippocampus and cingulate anterior cortex in rats during the training described above.

Modulation of Hippocampal Long-Term Potentiation by Holeboard Experience in the Rat. 660

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Long-term potentiation (LTP) is considered as a cellular model for learning and memory. We studied the impact of spatial learning on the maintenance of LTP in the rat dentate gyrus under the holeboard paradigm. In 8 week old rats a recording electrode was stereotactically and chronically implanted into the granular cell layer of the dentate gyrus and a stimulation electrode into the perforant path. LTP was induced by a weak

tetanization of the perforant path (3 bursts of 15 pulses, 200 Hz, 10 s interburst interval). Behavioral manipulations started 15 min after tetanization. Three groups of animals received a spatial training on a fixed pattern of baited holes of one, five and ten trials, respectively. The last trial was performed after tetanization. A control group was pretrained by nine trials with changing patterns of baited holes after each trial and then tetanized before starting the tenth trial. Rats significantly improved their spatial performance during the ten learning trials indicated by decreasing times to pick up all food pellets and by decreasing reference memory errors. LTP prolongation was significantly enhanced with increasing numbers of trials up to 24 h. In contrast, the control animals showed no LTP prolongation. The data provide further evidence that hippocampal LTP can be modulated by spatial learning. The impact of arousal and stress on the LTP modulation is currently under investigation.

661 High and low anxiety rats: Analysis of inhibitory avoidance behavior, pain reactivity, and the memory-modulating effects of a selective nicotinic agonist

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We have recently shown that male Wistar rats which are identical in stain, sex and age can differ systematically in their behavioral response to the elevated plus maze (EPM). There, subgroups of rats, termed high anxiety (HA) or low anxiety (LA) rats, can be identified based on the measure of time spent on the open arm. HA rats, which tend to avoid the open arms of the EPM, were found to have lower levels of ventral striatal serotonin (Schwarting et al. 1998), a transmitter which plays a critical role for anxiety (Graeff et al. 1996). Furthermore, they were also more anxious in an object burying task, and showed slower acquisition of active avoidance learning than LA rats (Ho et al. 2002).

Here, we asked if HA and LA rats also differ in an inhibitory avoidance task. Since behavior in active and inhibitory avoidance tasks is motivated by electric footshock, we also tested for possible differences between HA and LA rats in pain reactivity using a hotplate and a tailflick test. Furthermore, we examined the possible memory-modulating effects of the CNS selective nicotinic agonist RJR 2403.

In an inhibitory avoidance task of the step-in type, we tested three different shock intensities (0,15 mA, 0,3, mA and 0,5 mA) in three separate samples of animals. HA and LA rats did not differ in baseline step-in responding, nor in avoidance after shock experience. In the tail-flick test, there were also no differences between HA and LA rats. In the hot-plate test, we obtained indications for less pain reactivity in HA rats with repeated testing which may account for their inferior performance in the active avoidance test (Ho et al. 2002). Finally, preliminary results with the nicotinic agonist RJR 2403 showed that this drug can have dose-dependent amnestic effects (6µM, ip) when injected post-trially in the inhibitory avoidance task, an effect which was most prominent in HA rats.

This work extends existing knowledge in showing that important individual differences exist between normal male Wistar rats, which encompass behavior (motivation, emotion, learning/memory), physiology, and psychopharmacological reactivity. Taking such differences into account may not only help to set light upon usual data variability, but can provide a unique approach to examine brain and behavior in the animal model.

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Chronic application of the CRH-R1 antagonist R121919 enhances cognitive performance in mice

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In the past years a fundamental correlation between anxiety-related behavior and cognitive processes has been demonstrated. Some authors even argued cognitive dysfunction to be the primary presenting feature of pathological anxiety. Recently, we provided evidence that in two inbred mouse strains (C57BL/6 and DBA/2), which have previously been found to differ in their anxiety-related behavior, the degree of anxiety is differentially associated with enhanced performance of distinct informational processes (Ohl et al., in press). It was hypothesized that the enhanced anxiety-related behavior, which has been demonstrated in naive DBA mice, may be closely related to these differences in cognitive processing. Recent studies strongly support the hypothesis that dysregulation of the hypothalamic-pituitary-adrenocortical (HPA) system by an enhanced activity of CRH secreting neurons affect anxiety-related behavior and also cognitive processing. Recent findings in mouse models have indicated that the CRH 1 receptor (CRHR1) may be the primary target at which selective nonpeptide compounds should be directed. One of recently examined new compounds is R121919 developed by Neurocrine Biosciences (LaJolla, USA). R121919 binds with high affinity to cloned human and rat CRHR1 whilst binding to other receptors, known to be present in the central nervous system, is extremely low.

In the present study we investigated the high-affinity CRHR1 antagonist R121919 at both the behavioral and cognitive level in the two mouse strains by using the modified hole board (mHB). The animals were treated for 12 days, starting 5 days before an initial testing. During the last 5 days of the treatment schedule, the mice were cognitively tested in 4 trials daily in a visuo-spatial mHB task. The first results showed that 5 days of voluntary R121919-administration not only reduced anxiety-related behavior but also increased motivation in the initial test in both strains, contrasting the results of previous studies where acute treatment with R121919 affected exclusively the behavior in DBA/2 mice. In the visuo-spatial task, vehicle-treated DBA/2-mice displayed a better cognitive performance than vehicle-treated C57BL/6 mice. In both mouse strains a significant treatment-induced enhancement was found for cognitive processing. In addition, the treatment-induced cognitive changes were paralleled by changes in exploratory strategy

in C57BL/6 mice, while in DBA/2 mice the exploratory strategy remained unaffected. In summary, the results indicate that treatment with the CRH-R1 antagonist R121919 has both anxiolytic and cognitive enhancing properties thus emphasizing the hypothesis of a close interrelation between anxiety and cognition.

663 Functional relevance of Wernicke's area in adult language acquisition

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Introduction To understand patterns of neural recruitment during recovery from stroke-related aphasia, we need information which areas are required for language acquisition in the general adult population. Transcranial magnetic stimulation (TMS) offers the unique opportunity to study the behavioral effects of "virtual lesions", i.e., producing a focal transient disruption of neural functioning. We used TMS to suppress cortical excitability in the vicinity of one of the classical language regions, i.e., Wernicke's area (Cp5 of the international 10-20 EEG system), while healthy subjects were trained in an artificial language. This aimed to determine the functional relevance of Wernicke's area in adult language acquisition.

Methods Eight subjects (5 females; mean age: 27 ± 4 years; all left-language dominant for language as assessed by functional transcranial Doppler ultrasonography) were trained twice for five consecutive days, once under true stimulation (verum) and once under placebo stimulation (sham) conditions. Subjects learned different versions of an artificial lexicon for verum and sham conditions; the sequence of conditions and versions was randomized. The artificial language training involved forced-choice correct/incorrect statements to pseudoword-object pairings, which varied in statistical properties of co-occurrence (Breitenstein & Knecht, 2002). In a "transfer" test after the last training session, subjects had to translate the pseudowords into their native language. TMS was applied with a of figure-of-eight magnetic coil for 20 min at a rate of 1 Hz and 110 percent of the subject s resting motor threshold (mean stimulator intensity: 57 ± 7 percent of 2 Tesla; Magstim Rapid) on each of the five training days, immediately prior to 20 min of training.

Results Verum stimulation compared to sham stimulation significantly delayed initial language learning (ANOVA with polynomial contrast analysis: interaction of condition x day, $F(1,7) = 6.30$, $p = 0.04$). The effect vanished, however, during the fourth training session, indicating that the brain rapidly compensated for the extrinsic neuromodulatory influcence. Overall, subjects with stronger left hemisphere dominance were more affected by verum TMS than subjects with a weaker lateralisation for language (Pearson $r = -0.60$, $p = 0.11$).

Discussion The data provide evidence for a functionally relevant recruitment of Wernicke's area during language acquisition in adults. The rapid behavioral adaptation to the

TMS effects indicates that other areas compensated for the suppressed neuronal activity within Wernicke's area. One such area may be the Wernicke homologue in the right hemisphere. The implication for aphasia therapy is that recovery of semantic language functions is possible after destruction of Wernicke's area, at least when an associative learning approach is applied.

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Homeostatic regulation of synaptic strength in CA1 pyramidal neurons? 664

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Do principal neurons have a mechanism for preventing too many synapses from being potentiated? The desirability of such a mechanism is, first, that if too many synapses were potentiated, a neuron would be at risk of overexcitation; and second, selective differences between synapses would diminish, thereby reducing the information-storage capacity of neuronal circuits (Moser et al, Science, 1998). One might therefore expect some homeostatic mechanism to be in place that, after a certain threshold of overall potentiation is reached, limits further synaptic strengthening even if LTP induction criteria were met at individual synapses. As early LTP induction and expression is determined by local synaptic rules, the putative homeostatis may be somatic, necessitating studies of both early- and late-LTP to investigate this issue. Turrigiano et al (Nature, 1998) have established in cell culture that there is a homeostatic mechanism operational over a timeframe of days. A shorter timescale may be necessary, even for late-LTP, to prevent overexcitability.

Extracellular and intracellular electrophysiological recordings were made in acute, rat hippocampal slices for up to 8 hrs. To our surprise, the experiments do not yet provide support for the homeostatic hypothesis. We observed that, one hour after repeated tetanic induction of strong and long-lasting LTP in one Schaffer collateral pathway (S1), hippocampal CA1 neurons were still able to exhibit LTP in a second independent pathway (previously unpotentiated synapses, S2) in the same slice. Neither the amount nor the persistence of this LTP differed from the LTP induced in naïve control slices that had not sustained saturating LTP induction on an independent pathway. Early- and late-LTP did not differ.

Questions remain about the proportion of an individual neuron's excitatory input that is activated by stimulation of a single pathway. However, for the stimulation and recording paradigms used to date, we find no evidence for homeostatic regulation of LTP in adult neurons.

665 Behavioural analysis of mice expressing a PKG inhibitory peptide in cerebellar Purkinje cells

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In an attempt to find out more about the myriad signal transduction pathways that are involved in cerebellar LTD, we have devised a strategy to selectively block cGMP-dependent Protein Kinase function in Cerebellar Purkinje Cells. For this purpose we have generated transgenic mouse lines expressing a peptide inhibitor, selective for cGMP–dependent Protein Kinases under the control of the Purkinje Cell specific promotor L7/Pcp2. Microiontophoretical application of this peptide in slices of the cerebellar vermis of FBN/N wild-type mice has shown a partial reduction of LTD.

Out of seven transgenic mouse lines, two were chosen based on the levels of expression as estimated by Northern Blot analysis. These lines were bread to homozygosity and tested on a variety of behaviour paradigms such as the accelerating Rotorod or the Catwalk test.

For one of the lines the homozygous population displays a decreased capacity to adjust to the speeding rotation of the rod, as measured by shorter time on the rod, when compared to both wild-type or heterozygous animals. These animals will be used in an eye-blink conditioning paradigm to check their suitability as models for deficient motor learning and memory

666 Biophysical Evaluation of a Linear Model for Temporal Sequence Learning: ISO-learning revisited

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The goal of this work is to relate the ISO-learning (Isotropic Sequence Order) rule to the biophysical effects of spike-timing dependent plasticity (STDP) at a single neuron. The ISO-learning (Porr and Woergoetter, 2002, 2003) is a linear, reward-free algorithm for temporal sequence learning based on a differential Hebbian learning rule. This algorithm reproduces the typical asymmetrical weight change curve normally observed during spike-timing dependent plasticity (STDP) but it is based on rate-coded (time-continuous) and not spiking inputs. We show that the components of ISO-learning can be associated with a state-variable description of the neuronal biophysics of STPD. One central aspect of ISO-learning is the employment of band-pass filter operations before summing the inputs at the neuron. Band-pass filtering takes place in a natural way in a biophysical model as the result of the pre-synaptic (NMDA/AMPA) channel characteristics as well as the general (post-synaptic) membrane properties. We investigate the

influence of different possible sources of post-synaptic depolarization on the learning. Specifically we distinguish between other synaptic inputs versus the influence of a back-propagating spike (BP-spike) on the plastic synapse under considerations. In general we find that the different possible shapes of pre- and post-synaptic signals will strongly influence the shape of the weight change curve. We arrive at four main observations: 1) The obtained results suggest that the influence of a back-propagating spike is most likely responsible for driving the learning, because other synaptic inputs are (within physiological conditions) either too short of too weak. 2) The biophysical variant of the ISO-learning rule is linear over wide ranges only when using a BP-spike as post-synaptic depolarization source. 3) A possible influence of a tonic membrane depolarization (i.e., shifting the resting potential to influence the NMDA-channels) is moderate given that a BP-spike will normally lead to a much stronger (transient) depolarization anyway. 4) Differential Hebbian learning can turn to plain (symmetrical) Hebbian learning without having to change the learning rule as soon as the rising flank of the BP-spike becomes less steep which could be especially the case a distal dendritic synapses. In conclusion, this study attains an intermediate, descriptive level of the biophysics of STDP. The obtained results can mainly be used to implement the richness of different weight change characteristics without having to deal with the complexity and computational expense of kinetic models.

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Neuronal recruitment in adult zebra finch brain during a reproductive cycle

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The occurrence of neurogenesis (birth of new neurons) and new neuronal recruitment in adult brains is by now a well known phenomenon, found in several warm-blooded vertebrates, including humans. The hypothesis is that this phenomenon serves adaptive functions and contributes to the cellular basis of brain plasticity and ability to acquire new long-term memories. Accordingly, regulation of new neuron survival by extent of circuit use may be a general mechanism for ensuring that neuronal replacement is closely attuned to environmental change and to the need to store novel information.

Previous studies in our laboratory support this hypothesis and show that in adult birds, increase in information load is associated with an increased recruitment of new neurons: when adult birds are exposed to a new complex social setting, more neurons are recruited in parts of their brains that process auditory and visual information, than in brains of other birds which were exposed to a simple setting. These findings indicate that social complexity might affect neuronal survival.

Along this line, the present work is designed to further test the hypothesis that times when new and heavy memory loads have to be handled coincide with the recruitment of new neurons. To do this, we use an estrildid songbird - zebra finch (*Taeniopygia gutta-*

ta) - as a model system. This species is highly social and in nature it nests colonially and forages in large flocks of up to several hundred individuals. Nestlings remain in the nest for about two weeks and during this period they depend exclusively on their parents. After fledging when the young birds leave the nest and join with other juveniles in the colony, their parents keep feeding them for two more weeks until they reach independence. This suggests that prior to this stage parents must learn to recognize their offspring. Recognition can be elicited via visual as well as auditory or even olfactory cues. The hypothesis to be tested is that changes in hormonal profile during egg laying, incubation or rearing of the young, and attendant changes in experience, may trigger a sharp increase of new neurons into relevant parts of the brain, to enable parents-offspring recognition when the young have to be fed until they reach independence.

To do this, we inject adult breeding birds (males and females) with [3]H-thymidine, a cell birth marker, during different stages of their reproductive cycle, as follows: 1. control: birds are treated and then kept singly; these birds do not engage in reproduction. 2. birds are treated and two weeks later are paired and allowed to breed. 3. birds are paired allowed to breed and treated after the last egg is laid. 4. bird are paired, allowed to breed and treated when their juveniles fledge from the nest. Forty days later we record the number of new neurons in two brain regions: neostriatum caudale (NC; a forebrain region that processes auditory and visual information) and the olfactory bulb. A comparison between experimental groups will be presented and discussed.

668 The effect of cumulative experience on the use of elemental and configural visual discrimination strategies in honeybees

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We addressed the question of whether the amount of individual experience determines the use of elemental or configural visual discrimination strategies in free-flying honeybees Apis mellifera. We trained bees to fly into a Y-maze to collect sucrose solution on a rewarded stimulus presented in one of the arms of the maze. Stimuli were colour disks violet (V), green (G) or yellow (Y), which were of equal psychophysical salience for honeybees. Training followed an A+, BC+ design, followed by an AC vs. BC test. Training consisted of 6 (3 A+ and 3 BC+), 20 (10 A+ and 10 BC+) or 40 (20 A+ and 20 BC+) acquisition trials. Elemental models of compound processing predict a preference for the non-trained stimulus AC while configural models predict a preference for the trained stimulus BC. Our results show that increasing the number of acquisition trials results in a change of the internal representation of stimuli, visible through a change from elemental to configural choice strategies. After 6 training trials, bees favoured an elemental strategy and preferred AC to BC during the tests. Generally, increasing the number of training trials resulted in an increase of the choice of BC. Additionally, we observed that the change in stimulus processing was also influenced by stimulus similarity. Colour perceptual similarity favoured configuring with increasing experience.

Antennal tactile learning in the honeybee: Memory dynamics and effect of nicotinic antagonists 669

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Restrained worker honeybees (*Apis mellifera* L) are able to learn to extend their mouthparts when scanning a little metal plate with their antennae. Here, we describe the memory course and the effects of nicotinic antagonists on this antennal tactile learning. Acquisition could occur reliably in one trial and memory persisted 1 day after. Multi-trials acquisition (either distributed or massed) did not improve the retention level. The involvement of nicotinic pathways in this learning was tested by injecting either mecamylamine (0.6 μg per bees) or α-bungarotoxin (2.4 ng per bees) in the whole bee brain through the median ocella. To assess the activity duration of the drugs, we injected it 20 min after a single trial learning, and then we tested the animals at various time after this injection. This injection was preceded by a first recall test (pre-injection test). Therefore, we compared the performance between pre-injection test and the post-injection test, and the post-injection tests' performance of drug-injected animals and ringer-injected animals. Mecamylamine impaired the recall performance during 20 minutes, and it had no more effects 3 hours after the injection. Then, we performed a second experiment during which a single learning trial was followed by recall tests 1 hour, 3 hours or 1 day after. Mecamylamine injected 10 minutes before these recall tests impaired the recall performance. Furthermore, these effects could not be explained by an influence of mecamylamine on reconsolidation processes. When the mecamylamine was injected 10 minutes before a single trial learning, the 3 hours recall performance was impaired, which indicates that this drug blocks the acquisition. A single trial acquisition immediately followed by a mecamylamine injection did not impair the recall, showing therefore that mecamylamine does not affect the consolidation processes. As these results are consistent with previous studies on olfactory learning, nicotinic pathways seem to be involved in general memory in the honeybee rather than in olfactory specific memory. By contrast, bungarotoxin had no effects at all during the same experiments. However, injected 10 minutes before a five-trial acquisition, mecamylamine had no more effects on the 1-day recall performance, whereas in the same condition, bungarotoxin inhibited only the one-day recall. Hence, there is a pharmacological dissociation between honeybee medium term memory (triggered by one trial learning, mecamylamine sensitive) and long-term memory (triggered by multi-trial learning, bungarotoxin sensitive).

670 The effect of cumulative experience on the use of elemental and configural visual discrimination strategies in honeybees

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We addressed the question of whether the amount of individual experience determines the use of elemental or configural visual discrimination strategies in free-flying honeybees *Apis mellifera*. We trained bees to fly into a Y-maze to collect sucrose solution on a rewarded stimulus presented in one of the arms of the maze. Stimuli were colour disks violet (V), green (G) or yellow (Y), which were of equal psychophysical salience for honeybees. Training followed an A+, BC+ design, followed by an AC vs. BC test. Training consisted of 6 (3 A+ and 3 BC+), 20 (10 A+ and 10 BC+) or 40 (20 A+ and 20 BC+) acquisition trials. Elemental models of compound processing predict a preference for the non-trained stimulus AC while configural models predict a preference for the trained stimulus BC. Our results show that increasing the number of acquisition trials results in a change of the internal representation of stimuli, visible through a switch from elemental to configural choice strategies. After 6 training trials, bees favoured an elemental strategy and preferred AC to BC during the tests. Generally, increasing the number of training trials resulted in an increase of the choice of BC. Additionally, we observed that the change in stimulus processing was also influenced by stimulus similarity. Perceptual similarity between stimuli favoured configuring with increasing experience.

671 Properties of sensory neuron synapses in the trigeminal and auditory startle pathway

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The mammalian startle response is an excellent model for studying the cellular mechanisms of behaviour and learning. The primary neuronal pathway mediating this response is well understood, however, there are still some points to be clarified. We here investigated where and how sensory input from different modalities converge within this pathway and we further characterized plasticity of trigeminal and auditory synapses of afferent neurons.

Giant neurons in the caudal pontine reticular nucleus (PnC) represent the sensomotoric interface of the mammalian startle response. They receive short latency input from auditory fibres (mediating acoustic startle) arising from the cochlear nucleus or cochlear root. We here explored whether the same neurons receive also trigeminal input (media-

ting tactile startle). Synaptic connections between the principle nucleus of the 5th nerve (Pr5, mediating trigeminal signals) and PnC neurons could be shown on a cellular level by fluorescent tracing studies and whole cell patch clamp recordings in rat brain slices. Presynaptic stimulation of PnC giant neurons in the Pr5 revealed short latency glutamatergic EPSCs in PnC giant neurons which are mainly AMPA receptor driven. Paired pulse stimulation revealed strong facilitation. At an interstimulus interval (ISI) of 50ms EPSC2/EPSC1 ratio was 1.78 (±0.14 SEM, n=18). With increasing ISI, paired pulse facilitation (ppf) declined and at an ISI>200ms no more ppf was observed. Thus, short-term plasticity of trigeminal afferents resembles that of auditory afferents. Further, stimulation by repeated bursts at 1Hz in the Pr5 induced synaptic depression (n=9).This form of plasticity had been described before for auditory afferents and was proposed to underlie habituation of the startle response. Synaptic depression in the trigeminal pathway was slightly weaker than described for auditory inputs, which parallels a weaker habituation of the tactile startle compared to acoustic startle.

We further showed that auditory and trigeminal stimuli always converged to the same PnC giant neuron. The combination of trigeminal and acoustic stimulation led to a strong, nearly linear summation of the evoked EPSC (n=23), indicating that synapses of different modalities are not located next to each other on a dendrite.

Synaptic depression of auditory and trigeminal synapses did not interfere with each other, which is in accordance to behavioural studies that demonstrated a lack of interference of habituation of the acoustic and the tactile startle response. It further indicates either a purely presynaptic mechanism for synaptic depression or the location of synapses on distinct dendritic compartments of the PnC neuron.

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How is the egocentric spatial orientation represented in the striatum? 672

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The cause of Parkinson's Disease is the degeneration of dopaminergic neurons. The neurons projecting from the midbrain to the dorsal striatum are mainly affected, and the resulting dopamine-deficiency is predominantly expressed in the basal ganglia. The main symptoms of Parkinson's Disease are motor disturbances but also disturbances of cognitive performance like habit-learning. The question to be answered here is whether the motor disturbances are due to the same mechanisms that underly disturbed habit-learning. In general this may indicate that motor behaviour and habit-learning are controlled by the same mechanisms and these mechanisms are dopamine-dependent. With these experiments we examined the effects of dopamine deficiency on the electrophysiology of medium sized spiny neurons of the striatum in connection with observable changes in habit-learning. The observed habit was the acquisition of a procedural representation of the egocentric spatial orientation.

In these experiments we recorded extracellulary in the striatum with a sample frequency of 32 kHz. The electrodes were implanted into the striatum of male Spague-Dawley rats. The rats could move freely during the experiment. The electrophysiological recordings were performed during a delayed alternation task in a T-Maze (Hauber, 1989). The subjects were divided into two groups: a control group, which was injected with Saline and a test group, which was injected with 0.1 mg/kg of the D2 receptor antagonist Haloperidol.

The test group showed a different electrophysiological activity compared to the control group. The spike activity in haloperidol-treated animals was significantly lower (Two way ANOVA, $p < 0.01$) than in the control group. Also, the burst rate (bursts per second) were significantly higher (Two way ANOVA, $p < 0.01$) in the control group than in the haloperidol group. During the experiment we could observe that some of the animals showed akinesia despite the low concentration of haloperidol. The haloperidol group was divided into a group of akinetic animals and a group of non-akinetic animals. Akinetic and non-akinetic subjects differed significantly in terms of burst rate with the latter showed a higher burst rate than the aktinetic animals. Another critical variable is the accuracy in the T-Maze. The animals of the control group, which have performed correct showed a higher amount of bursts than the other animals (T-Test, $p = 0.007$).

In conclusion, we think that (1) the manipulation of the D2 receptors with haloperidol shows effects on the electrophysiology of the striatal neurons, (2) the different burst rates of the akinetic and non-akinetic subjects let us assume that burst firing is not dependent from pharmacological treatment but from the behaviour exhibited and (3) that the burst rates are correlated with the right or wrong accomplished task. So we assume that the burst hold information that are relevant for the egocentric orientation task.

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673 Role of group III mGluR in synaptic depression in the PnC

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Giant neurons in the PnC (nucleus reticularis pontis caudalis) play a central role in the acoustic startle reaction (ASR) of the rat. We previously suggested that homosynaptic depression (HSD) in the PnC, inducible by repeated stimulation of auditory afferents in a brainstem slice preparation, is most likely the cellular basis for a relatively simple form of learning, namely the short-term habituation of the ASR (Weber et al., Europ. J. Neurosci. 2002). Thus, studying the cellular processes of HSD in the PnC in comparison with behavioral experiments of the ASR brings together cellular mechanisms and behavioral learning in mammals.

HSD in the PnC is inducible by stimulation of auditory fibers that arise from the cochlear nucleus (100 bursts at 1Hz). Voltage-clamp recordings of giant neurons in the PnC

revealed an exponential decay of evoked EPSC magnitudes with a total reduction of 52% (Weber et al., Europ. J. Neurosci. 2002). We found strong evidence for metabotropic glutamate receptors (mGluRs) of group III mediating HSD: bath application of MPPG (200µM, n=6) and CPPG (100µM, n=6), both mGluR III antagonists, blocks HSD induction (only 1% and 19% total reduction of EPSC magnitudes, respectively). On the other hand, L-AP4 (50µM, n=6), an mGluR III agonist, strongly reduces synaptic efficacy (about 70%) and occludes depression by subsequent repeated stimulation (100 bursts at 1Hz). Similar effects can be observed with DCPG (1µM), a specific mGluR8 subtype agonist (preliminary data: 53% reduction of synaptic efficacy, n=2). These pharmacological data indicate an important role of mGluR III in HSD induction, in particular subtype mGluR8.

We have further evidence for the involvement of subtype mGluR7 in HSD induction: giant neurons in mGluR7 knock-out mice (C57 strain) show a clear reduction in HSD. Whereas EPSC magnitudes of giant neurons in homocygote wild-type mice decrease by 49% (n=6), EPSC magnitudes of homocygote mGluR7 knock-out mice decrease only by 32% (n=5) with a much slower time constant. HSD in heterocygote mice is slightly stronger than in the mGluR7 knock-out animals (36% total reduction of EPSC magnitudes, n=20), but the time constant is as fast as in the wild-type animals.

The role of another mGluR III subtype in HSD, mGluR4, will be tested in future with mGluR4 knock-out mice.

We conclude that group III mGluRs are the major component in mediating HSD in the PnC. These receptors are probably located presynaptically (Weber, unpublished data) functioning as autoreceptors that turn down strong synaptic activity. The subtype mGluR8 seems to be most important, while subtype mGluR7 seems to play a minor role. Further investigations will clarify the involvement of other mGluR subtypes, especially mGluR4, in HSD induction.

Associative learning in individually assayed *Drosophila* larvae 674

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One of the key preparations for the study of synaptic plasticity is the *Drosophila* larval neuromuscular synapse. However, progress to link the processes discovered at the synaptic level to behavioral plasticity has been limited because of the lack of a reliable behavioral learning paradigm for the *Drosophila* larva. We sought to overcome this limitation by introducing two new associative learning paradigms, both of which use individually assayed *Drosophila* larvae. In one paradigm, larvae are trained to associate odorants, in the other visual stimuli, with differential gustatory reinforcement. We compare individuals which have undergone either of two reciprocal training conditions: For example, odors may be paired with either fructose ("+") or quinine ("-"). In one training

condition, individuals are trained by pairings of odorant A with "+" and odorant B with "-" (A+/ B-); in the reciprocal training condition, individuals are trained A-/ B+. In the test phase, individual animals are given a choice between the two odors and their preferences are measured. A difference in choice between A and B of individuals having undergone A+/ B- versus A-/ B+ training thus unequivocally indicates associative learning. Such associative learning can indeed be demonstrated in both the visual and the olfactory version of this training paradigm. We now take the first steps towards an understanding of the molecular requirements for these two kinds of learning. Specifically, mutations in the type-I adenylate cyclase gene (rut2080) and in the synapsin gene (syn97) (see also contribution by Diegelmann et al.) are investigated. As the established paradigms use individually assayed larvae, subsequent analyses at the physiological level should also be facilitated.

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675 A new olfactory learning paradigm for single flies in the flight simulator

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The mushroom bodies (MBs) of *Drosophila melanogaster* are required for olfactory but not visual learning and memory(1). On the other hand, visual memory at the flight simulator can be blocked by presenting the training and the memory test in different contexts (e.g. different colors of the background illumination) and the stability of the memory trace with respect to such context changes (context generalization) is strongly reduced in flies with defective or missing MBs(2).

Olfactory learning has so far been tested mostly under conditions involving massive but methodically unavoidable changes of context between training and test(3). This raises the question whether in olfactory learning the mushroom bodies might be required primarily to stabilize the memory trace against the context change during transfer from the training tube to the test tube.

To investigate this possibility, we designed an olfactory learning experiment according to the protocol of the visual experiment in the flight simulator. The visual cues (upright T's vs. upside down T's with different heights of their centers of gravity) are replaced by two different odor stimuli (e.g. Octanol and 4-Methylcyclohexanol). Flies stabilize their flight with respect to the visual panorama which has no landmarks. They can choose their flight orientation according to the odors and can be trained to avoid one of them. As in the visual learning task, training and test procedures can be performed with or without changes of the context. Special attention will be paid to the role of the mushroom bodies in this new paradigm.

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Effects of reinforcement on the activity in areas 17 and 21A in the alert cat 676

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Representations in primary sensory areas are often thought to passively reflect properties of the environment, and, in the adult, show limited plasticity. In the present report, we study neuronal activity in area 17 and 21a in alert cats during a reinforcement task. Training on a Go/noGo task consists in a bar pressing during the presentation of a moving grating of a specific orientation. When performance is stable, movable tetrodes are implanted and recordings are performed head fixed. The response strength of the local field potential is characterized by the relative change of the spectrogram (200 ms after stimulus onset) compared to the blank screen. We find stimulus induced activity mainly in the γ-frequency range. For both areas 17 and 21a, we find two peaks (~55 Hz and ~100 Hz) and a decay at higher frequencies (13% at 150Hz). Reinforcing one orientation leads to a twofold increase of the stimulus-induced response. This increase is independent of the preferred orientation (measured with gratings of higher spatial frequency). This effect is stable over days and is observed for test stimuli even outside of the task proper, making a general attentional effect unlikely. During the reinforced stimulus, the coherence between area 17 and 21a increased between 40 and 80 Hz (~15 %) whereas, during the negatively reinforced stimulus, the coherence decreased (~-30%) at two minima (~55 Hz and ~100 Hz). During reversal, at the same recording site, the previously reinforced stimulus decreased its modulation, and after a week (~400 stimulus presentations), reaches equivalent level to the other stimulus. These results demonstrate that neuronal activity includes surprisingly high frequency components and is strongly modulated by reinforcement in higher and even in primary visual cortex. This reinforcement also influences relations between these areas. Furthermore, this modulation is sensitive to the validation of the reinforcement.

Common design in brains of velvet worms and chelicerates and their phylogenetic relationships. 677

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Immunocytological studies on the brains of the onychophoran *Euperipatoides rowellii* and araneans suggest major similarities of columnar neurons and layering in a superficial midline neuropil known as the arcuate body (see accompanying poster: Loesel and Strausfeld, 2003), which is synapomorphic to all chelicerates including the Pycnogonidae. Observations on onychophorans and chelicerates (*Limulus*, solfugids, uropygids)

show that these also share detailed neuronal arrangements in their corpora pedunculata and in glomerular olfactory lobes. Furthermore, projections from the second optic neuropil of the primary visual system of *Limulus* to the lateral neuropils of its midline neuropil, a feature that is unique to the chelicerates, also characterizes connections from the second optic neuropil of the single lens eye of *Euperipatoides* to its arcuate body analogue. Although externally onychophorans appear quite different from chelicerates, this may be misleading. In juvenile *Limulus*, as in onychophorans, the ventral nerve cord is lateralized and throughout its length contains synaptic neuropil. Further, the segmented embryos of chelicerates are arguably reminiscent of postnatal onychophorans. Phylogenetic analysis using parsimony (PAUP) employing over 100 discrete neural characters recorded as present or absent in 42 taxa representing all the major arthropod groups suggests that the Onychophora are allied, and basal, to the chelicerates. These findings further imply that chelicerate-like lobopod arthropods appeared earlier in deep time than the crustaceomorphs, diplopods, and chilopods although the early fossil record shows these groups as co-existing. The present findings further provide novel insights into the organization of the onychophoran nervous system and the phylogenetic relationships of this arthropod group.

678 The morphology of descending dorsal unpaired median (DUM) neurons of the locust suboesophageal ganglion

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From the suboesophageal ganglion of locusts 6 neurons with dorsally located somata send bifurcating axons into the ganglia of the ventral nerve cord (Bräunig, 1990, Phil. Trans. R. Soc. B). The axons of at least some of these neurons descend as far as the terminal ganglion. It is not known whether these 6 cells form a group of 6 individual neurons where each type has a distinct morphology. For this reason individual somata were penetrated with microelectrodes and stained by iontophoretically introducing cobalt hexammine chloride or neurobiotin into the cell. In most cases the ramifications were completely stained in the suboesphageal and the first thoracic ganglion. In exceptionally well stained cases also the ramifications in the mesothoracic ganglion were stained. Within the thoracic ganglia the neurons ramify profusely, also invading ventral (sensory) neuropiles. Evaluation of some 60 stained cells allowed a classification of these neurons into only three distinct morphological types. This raises the question whether these neurons might be paired neurons, although judged by all other criteria (such as dorsally located somata, bifurcating axons, soma spikes, octopamine-like immunoreactivity) they are members of the class of dorsal unpaired medien (DUM) neurons.

Topographic representation of sensory signals in the thalamus of the fire bellied toad (*Bombina orientalis*) 679

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The function of the amphibian thalamus and its putative homology to particular thalamic subnuclei in amniotes is a matter of long debate. Based on anatomical data and on comparative studies, the amphibian dorsal thalamus is considered as an important relay center for sensory information. However, due to its specific input-output relationship the amphibian ventral thalamus suits much better as a candidate for a multimodal thalamic relay center. Specific data for neurons in the amphibian thalamus exist mainly for visually driven units while anatomical data for other sensory systems indicate that rather the ventral thalamus relays sensory signals towards the telencephalon.

As a first step to localize a putative equivalent of the amniote thalamus in amphibians, we studied the topographical representation of multimodal sensory signals in the diencephalon of the fire bellied toad *Bombina orientalis*. Experiments were carried out on isolated brains of 6 specimens. Electrical stimulation of cranial and spinal nerves was performed by applying single constant current pulses across suction electrodes. The Vth nerve, the anterior ramus of the VIIIth nerve and the dorsal root 3 and 8 were stimulated bilaterally with respect to the recording site. The spatial distribution of cranial nerve and spinal dorsal root-evoked field potentials in the thalamus was systematically mapped with 16 dorso-ventral depth tracks through the diencephalon organized in four frontal planes. Since field potential amplitudes showed stimulation site-specific depth profiles, two-dimensional activity maps for each of the four frontal planes were used to evaluate the differential spatial distribution of responses evoked from different sensory nerve roots.

In general, the largest amplitudes after stimulation of any sensory nerve root were found within the ventral, central and posterior thalamus. However, a distinct topographical organization for responses evoked from different stimulation sites was encountered in these areas. Responses evoked from the Vth nerve and spinal dorsal roots 3 and 8 occupied more or less separate thalamic regions, whereas responses evoked from the vestibular organs overlapped in part with those of the Vth nerve or spinal dorsal roots 3 and 8. These results suggest that information from different sensory origins is processed in topographically separate regions within the amphibian thalamus as it is the case for the amniote thalamic nuclei. However, these results also indicate that the dorsal thalamus plays a minor role as a recipient area for different sensory inputs and thus might not be directly equivalent to the amniote thalamus. Rather the dorsal part of the thalamus in amphibians might be involved in limbic functions. Future studies determining the precise structure-function of thalamic neurons, its synaptic inputs from different sensory nerve roots and its precise location within the amphibian thalamus will show if there are in fact anuran equivalents of particular mammalian thalamic subnuclei.

680 Histological and Immunocytochemical Evidence for a Metasomal Light Sense in Scorpions

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Searching for the eyes of the circadian clock, extraretinal photoreceptors have been proposed as putative Zeitgeber receptors in several invertebrate and vertebrate species. Only in few cases all three aspects, structure, physiology and chronobiology, could be demonstrated in matching experiments. In scorpions a metasomal light sense has been shown by electrophysiological measurements (e.g., Geethabali Diss). But the structure and localisation of these photoreceptors are unknown, so far.

By immunocytology with various antibodies against histamine and proteins of the phototransduction cascade (arrestin, opsins) a few cells in the sixth and seventh abdominal ganglia of scorpions could be marked. They lie in little clusters of 3-4 cells. Their outgoing nerves end in a nearby tiny neuropil, which is contacted by histaminergic interneurons. In the same ganglia, next to these histaminergic pathways, we detected serotonergic and glutaminergic neurons, which seem to make mutual contacts. The functional meaning of this network is still unknown.

The ultrastructure of the putative receptor cells provides further evidence for a photosensitive function: rhabdome-turnover products and rhabdomeric microvilli, which change with light/dark adaption, single intracellular cilia or basal bodies, and efferent synaptic contacts.

Now we can investigate their meaning as a component part of the complex natural Zeitgeber receptor machinery.

681 Long Range Intrinsic Connections in Human Motor Cortex

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Most cortical areas display a modular organization of their functional architecture. Different functionally cooperating modules are often linked by long range intrinsic axons in the supragranular cortical layers. So far, these features have been demonstrated mainly in sensory and association areas, but little is known about the intrinsic connectivity in the motor areas. Therefore, we have analyzed the organization of long range intrinsic connections in the human primary motor cortex using post mortem tracing with carbocyanine dyes in 4 cases. These examinations revealed the presence of long range intrinsic axons in particular in supragranular layers ranging up to 10mm. Besides anterogradely labelled axons there were also retrogradely labelled cell bodies. The connections were topographically organized forming clusters about 800-900 µm in diameter at regularly spaced intervals around the injection sites. In these clusters, both, anterogradely and retrogradely labelled neuronal elements could be detected suggesting a recipro-

cal nature of these connections. In several cases the connectivity patterns were not symmetrically distributed around the injection sites, but exhibited a tendency to preferentially extend along certain trajectories. Our results indicate that in human primary motor cortex a network of long range intrinsic connections reciprocally binds spatially separated clusters of neurons in the supragranular layers. It can be speculated that these connections may underly the coordination of the activity of spatially separated representations of muscle groups or motion vectors which synergistically interact for the execution of complex movement patterns.

Diffusion tensor MR imaging: Preliminary applications to mice, rats, and squirrel monkeys

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Progress in neurological animal models rises the demand for non-invasive imaging methods to detect axonal integrity, extent of myelination and neuronal connectivity in the brain of laboratory animals. Based on the anisotropy of water diffusion, mapping of the diffusion tensor (DT) by magnetic resonance imaging (MRI) in humans revealed local fiber orientations and even distant projections of axonal fibers [1]. So far, applications of DT-MRI in experimental animals *in vivo* are hampered by the requirement of smaller voxel sizes which results in a relatively low signal-to-noise ratio (SNR) at reasonable measurement times. The purpose of this study was to evaluate the potential of a novel half-Fourier (HF) DT-MRI sequence (STEAM) for diffusion studies in mice, rats, and squirrel monkeys *in vivo*.

The HF DT-MRI sequence (STEAM) [2] was implemented on a 2.35 T Bruker DBX system with BGA20 gradients (100 mT/m). For DT MRI magnetic field gradients corresponding to a diffusion weighting of b = 200 s/mm^2, 700 s/mm^2, and 1200s/mm^2 (gradient pulse duration = 9.5 ms, diffusion time = 15 ms) were applied along 6 different directions. The total measurement time was below 3 h. Calculation of the DT was based on a multivariate regression analysis and allowed for mapping the relative anisotropy and the main diffusion direction.

Anesthesia, stereotactic device, and coil arrangement for signal excitation and data reception were optimized for each species. In mice, a spatial resolution of $140 \times 280 \times 720$ μm^3 sufficiently resolved the main fiber structures of corpus callosum, commissura anterior, and fimbria hippocampi. In the larger brain of rats a spatial resolution of $200 \times 300 \times 750$ μm^3 allowed the additional detection of internal capsule, external capsule and truncus medialis cerebri. In monkey brain a spatial resolution of $400 \times 700 \times 1000$ μm^3 revealed a comparable number of observable fiber tracts as reported in human studies [1].

Subsequent applications will deal with normal brain development and with the investi

gation of animal models of degenerative white matter diseases. Further insights are expected by using advanced fiber tracing methods, in particular in non-human primates.

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683 Mitochondrial network organization and motility in mouse respiratory neurons

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Mitochondria of brainstem neurons that were cultured from the isolated respiratory center (preBötzinger complex) of juvenile mice form a well organized dynamic network. Visualization of rhodamine 123- or CFP-labeled mitochondria using a custom-built two-photon laser scanning microscope (see our technical poster) revealed filamentous structures of up to 40 µm length predominating in cellular processes. Punctate structures or irregularly- shaped clusters were mostly found in the soma. Time-lapse recordings revealed a highly dynamic organization of this mitochondrial network. The single mitochondrial structures showed chaotic, Brownian motions corresponding to an apparent diffusion coefficient of $4x10^{-3}$ $\mu m^2/s$ or bidirectional shifts with an apparent diffusion coefficient of $50x10^{-3}$ $\mu m^2/s$ and a velocity of up to 0.4 µm/s. The latter type of movement occurred along the longitudinal axis of cellular processes, and it was characterized by several stop and go processes. Furthermore, directed mitochondrial movements were ATP-dependent, slowing down upon blockade of mitochondrial metabolism by FCCP or oligomycin. The organization of the mitochondrial network was, however, not affected by such treatment. Depolymerization of microtubules by colchicine and inhibition of protein phosphatases by calyculin A abolished all directed movements, disrupted the mitochondrial network, and caused a subsequent accumulation of mitochondria within the soma. Pretreatment with the microtubule-stabilizing agent taxol prevented the colchicin-induced withdrawal of mitochondria from neurites. Disruption of actin filaments by cytochalasin B reversibly arrested the directed mitochondrial movements, suggesting that microfilament breakdown may interfere with the transport of mitochondria along microtubules. Forskolin and IBMX also reversibly blocked the movements of mitochondria, but did not affect their spatial distribution. Thus, phosphorylation of the cytoskeleton and mitochondrial proteins seems to be crucial. Moderate phosphorylation apparently regulates the transition from motile to non-motile mitochondria, while hyperphosphorylation causes detachment of mitochondria from microtubules which obviously serve as both docking sites and tracks. The discontinuity, velocity and ATP-dependence of directed mitochondrial movements suggest an involvement of molecular motors running along microtubules. The close interaction of mitochondria with cytoskeletal structures may guarantee a constant adjustment of the mitochondrial network to the cell´s energy requirements: mitochondria are immobilized at energetic hot spots and rearranged in response to changes in local energy demands.

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Integration of growing local interneurons into the mushroom body system of mature cricket brains is reflected by structure 684

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The paired columnar mushroom bodies (MBs) of the insect brain represent a prominent neuropile for multimodal sensory integration, most important for the organization of olfactory behaviour, and for memory and learning. The MBs always contain a class of local interneurons, the Kenyon cells (KCs). In adult cricket brains - in contrast to other insects species - solely a group of KCs is continuously newly formed from persisting neuroblasts throughout adult life. These growing and differentiating neurons have to be integrated into a structurally established, functional synaptic network, which is indispensable for organizing olfactory behaviours.

In *Gryllus bimaculatus*, we study the sprouting KCs in the MB compartments in relation to differentiated MB neurons, using immuno light and conventional electron microscopy. A conic cluster of about 100 growing KCs, stemming from mitotic neuroblasts in the MB pericaryal layer, forms a fibre bundle intensely stained by anti-actin fluorescence marking descending to the anterior MB calyx. Glial elements in this region appear actin-negativ. In the calyx, the central growing KC fibre bundle forms radial actin-positive dendritic projections joining differentiated KC dendrites, crossing an established GABAergic network before terminating at the centripetal margin of the layer of cholinergic input relay neurons. The stained compact sprouting fibre bundle, occupying a tenth of the stalk volume and containing about 800 fibres, keeps a central position in the stalk and α-lobe and a latero-marginal site in the β-lobe, and remains free of anti-synapsin immunoreactivity. Laser scan microscopy of longitudinal sections reveals tiny centrifugal growth tips arranged at the margin of the growing fibre bundle. In search for synapsing non?KCs (extrinsic neurons), we investigated the relation of GABA-, 5-HT- and octopamine immunoreactive neurons to the growing KCs. Tiny immunostained fibres, indicating synaptic contact, mainly terminate at the lateral rim of the actin-positive central fibre mass, but rarely intrude it. Synaptic sites were detected in samples of complete thin sections of MB compartments by electron microscopy. The intense anti-actin staining of growing KC fibres corresponds to their enhanced electron density. Synapses adjacent to sprouting fibres appear small with few synaptic vesicles and weak presynaptic electron dense membrane appositions, differing from fully developed synapses of extrinsic fibres with KCs in surrounding centrifugal portions of the stalk and lobe neuropile. The formation of new synapses apparently involves growth of extrinsic cell types in addition to the sprouting of newly added KCs.

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685 Median nerve neurons in thoracic ganglia of the cockroach, *Periplaneta americana* L.

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The unpaired median nerve (MN) and the associated transverse nerves (TNs) which contain the segmentally arranged perisympathetic organs (PSOs) play an important role as neuroendocrine centers in thorax and abdomen of insects. However, the neuronal organization of the MN/TN system is less investigated, especially the one which is linked to the thoracic ganglia. In *Periplaneta americana* L., 3 cell clusters with processes into the MN were stained in the abdominal PSOs by nickel backfills. These clusters correspond to the immunostained neurons[1, 2]. In the present study, I identified the thoracic MN neurons with the backfill technique. Staining of a TN reveals up to 12 neurons in each thoracic ganglion lying all ventrally and show a bilateral symmetrical arrangement. Two large and small median cells (LMCs and SMCs) are located near the ventral midline and a group of 4 postero-lateral cells (PLCs) occurs on each side of the ganglion. The axons of these neurons bifurcate in the MN at the origin of the TNs. The LMCs (20-30 µm in diameter) have extented arborizations within the central neuropile and represent the 2 spiracular closer motor neurons. The SMCs are 10-15 µm in diameter and were not stained in the prothoracic ganglion (T1). The PLCs (20-30 µm in diameter) possess only one main axon trunk with branches in the antero-dorsal ganglion part. In the T1 they send also processes into the anterior MN. In addition, 2 posterior medio-lateral cells (PMLCs) were stained in the ventral part of the next anterior ganglion. The somata of these cells have a diameter of 20-30 µm and their axons project via the connectives. Filling of the TN of the first unfused abdominal ganglion stained 6 cells in the abdominal neuromere of the metathoracic ganglion. 2 cell pairs are situated near the posterior midline and one cell lies on each postero-lateral edge of the ganglion. The described median and medio-lateral cells of the MN in the thoracic ganglia are motor neurons whereas the PLCs presumably contain neuropeptides which differ from those of the abdominal neurohemal organs or retrocerebral complex[3].

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Chemoarchitecture and *in vivo* labelling of cholinergic neurons in the rabbit basal forebrain

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The rabbit basal forebrain and its cholinergic components are frequently used targets to model neuropathological alterations associated with Alzheimer's disease. However, such studies are partly hampered by the lack of neuroanatomical data on the organization of rabbit basal forebrain cholinergic nuclei and their projections. Hence, serial coronal sections from rabbit basal forebrain were stained for choline acetyltransferase (ChAT) with an immunoperoxidase method that allowed quantification of cholinergic neurons in distinct nuclei and of fibre densities in defined neocortical and hippocampal areas. Consecutive sections were applied to the immunoperoxidase staining of low-affinity neurotrophin receptor p75 ($p75^{NTR}$) expressed by the vast majority of cholinergic projection neurons. In addition, carbocyanine double fluorescence labelling was employed to reveal the neurochemical heterogeneity of cholinergic neurons. The immunodetection of ChAT was combined with the detection of the neuronal markers $p75^{NTR}$, neuronal, nitric oxide synthase (nNOS), calbindin, calretinin, parvalbumin, tyrosine hydroxylase or substance P.

Cholinergic projection neurons were found in the medial septum/diagonal band complex, the ventral pallidum and the magnocellular nucleus basalis (MBN)/substantia innominata (SI) complex. All but SI cholinergic projection neurons co-expressed $p75^{NTR}$. Cholinergic interneurons were observed in the caudate nucleus, putamen, accumbens nucleus, olfactory tubercle, neocortex and hippocampus. While minor populations of cholinergic neurons in the MBN were immunopositive for nNOS and calbindin, all other investigated markers were only detected in non-cholinergic cells.

In vivo labelling with carbocyanine 3-conjugated anti-$p75^{NTR}$ (Cy3-ME20.4IgG) was established as a novel method to detect cholinergic projection neurons not only in fixed tissues, but also in living slices. Three days after intracerebroventricular injection, Cy3-ME20.4IgG selectively labelled cholinergic basal forebrain projection neurons. Future pharmacological studies with Cy3-ME20.4IgG-pre-labelled live tissues appear promising to unravel further cellular characteristics of cholinergic neurons.

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687 Medial prefrontal cortex of the Mongolian Gerbil: Anatomical subdivisions, thalamic connections, and auditory cortical afferents

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The rodent prefrontal cortex consists of orbital, agranular insular, and cingulate areas (CG1-3). Latter form, eventually together with the infralimbic area (IL), the medial prefrontal cortex (mPFC), which is, for instance, essentially involved in temporary memory including auditory working memory, spatial orientation, and behavioural flexibility.

Here we present results about the anatomical parcellation of the gerbil's mPFC, its major thalamic connections, and its afferents from auditory cortical areas.

To characterize the architecture of the mPFC we used staining methods for cell bodies (Nissl), myelinated fibers, acetylcholinesterase (AChE), cytoskeletal protein (SMI-32), and calcium-binding protein (PV). Thalamic connections were investigated by injections of the tracer biocytin into different areas of the mPFC. The auditory cortical input of the mPFC was evaluated by means of the tracers fluorescein- and tetramethylrhodamine-labeled dextran which were simultaneously injected into high- and low-frequency areas of the primary auditory cortical field AI, the anterior field AAF, and the posterior fields DP and VP, respectively.

In the Nissl stain the lamination of the mPFC has an agranular appearance with increasing laminar differentiation from CG2 to CG3 and CG1. Cells are most tightly packed in the deeper layers of CG3. Myelinated fibers are only sparsely distributed within the mPFC, mainly in layer II, and additionally in the deep layers of CG1 and CG3. Fibers stained for AChE form a complex network in the mPFC most entangled in CG2. The SMI-32 antibody recognizes primarily pyramidal cells, mainly in layers II/III and V/VI. This bilaminar staining pattern was most obvious in CG1 and CG3. The PV antibody labels mainly non-pyramidal neurons, particularly in layers II/III and VI of CG1.

IL is characterized by a diffuse distribution of cells and fibers and a weak staining for SMI-32 and PV.

Predominant targets of corticothalamic projections of CG1-3 are the mediodorsal thalamic nucleus (MD) and the rostral reticular thalamic nucleus (Rt). In addition, retrogradely labeled cells in the MD indicate reciprocal connections between MD and CG1-3.

Auditory cortical projections to the mPFC arise from all auditory cortical fields investigated so far. Projections from high- and low-frequency areas of AI are not tonotopically organized and converge strongest in CG3, IL, and CG2, as well as in CG1 and other surrounding prefrontal areas. Projections from AAF, DP, and VP are generally stronger and terminate in slightly different areas of the PFC than those from AI.

In summary, the cyto- and fiberarchitectural parcellation of the gerbil's mPFC corresponds to that previously reported for the rat. The use of antibodies against neurofilament and calcium-binding proteins revealed additional chemoarchitectural parcellation

patterns. The connections of CG1-3 with MD and rostral Rt prove their classification as prefrontal cortical areas. The multiple direct projections from auditory cortical areas to the mPFC demonstrate their substantial influence onto the processing of auditory information in the context of learning and memory.

Talairach-transformation and the localization of primary auditory cortex. 688

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Interindividual variability of the brain-anatomy is a main problem for a parcellation of the human auditory cortex. As an approach to this problem the Talairach transformation (Talairach and Tournoux (1988) Co-Planar Stereotaxic Atlas of the Human Brain, Thieme, Stuttgart) in combination with the coordinates of the Brodmann areas is used by many functional imaging studies. We tested the reliability of such an approach applied to the primary auditory cortex (Brodmann area 41). Therefore the 3D-dataset of the brain of ten subjects was Talairach transformed by using BrainVoyager 2000TM. We then determined the overlap between Brodmann area (BA) 41 and the area of Heschl's gyrus identified in each individual brain. On average 14.6% ± 11.2% of BA 41 was located on Heschl's gyrus on the left hemisphere and 4.6% ± 4.9% on the right hemisphere. Overall there was a strong tendency for posterior displacement of BA 41.

The results show that using the Talairach coordinates of BA 41 to describe the location of fMRI activation is misleading. In contrast, reliable landmarks (Heschl's gyrus and 1st Heschl's sulcus) allow to match the spatial fMRI patterns to individual anatomical variations.

3D Reconstructions of Pupal and Adult Glomeruli in the Antennal Lobe of the Sphinx Moth *Manduca sexta* 689

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Although the antennal lobe of the sphinx moth *Manduca sexta* is an established model for neurodevelopmental processes there is neither spatial nor quantitative information present about the development of the glomerular neuropil. The neuropil of the antennal lobe in *Manduca sexta* is organized into 63 glomeruli which are arranged in a sphere around a central coarse neuropil. Olfactory receptor neurons (ORNs) in the antennae carry the odor information into the antennal lobe. This information is integrated and processed in the glomeruli by local interneurons and sent to higher brain areas via projection neurons. Pheromone information is processed in three distally situated male-specific glomeruli (cumulus, toroid 1, toroid 2 or horseshoe) summarized as macroglomerular complex (MGC) [1]. The adult brain of *Manduca* is formed during 20 days of pupal development (P0 – P20). All cells of the antennal lobe are born at about P3. From

P4 the ORNs begin to enter the antennal lobe and lay out the matrices for the glomeruli. The interaction of ingrowing ORNs, glial cells, projection neurons and local interneurons mainly between P8 and P12 lead to the formation of the glomeruli. After P12 the glomeruli increase in size, but the main wave of synaptogenesis seems to be finished [2]. Here we compare for the first time 3D reconstructed glomeruli of whole antennal lobes of P13 males and pharate males (one day before eclosion) regarding to glomerulus position, size and shape. For glomerular labeling we used a monoclonal antibody against the ubiquitous synaptic vesicle protein synaptotagmin which reliably labels neuropilar structures [3]. Wholemounts of labeled isolated antennal lobes were imaged with a confocal laserscan microscope (Leica TCS SP2) and further processed and reconstructed with AMIRA 2.3 (TGS, San Diego, USA). Comparison of the antennal lobe reconstructions show that the glomeruli increase in size while retaining their relative position. Especially the glomeruli of the MGC enlarge about 100% in this period of time, while normal glomeruli increase only about another 20-30%.

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690 Immunoreactivity against octopamine in the pupal and adult brain of the honeybee, *Apis mellifera*

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We studied the arborisation pattern of octopaminergic neurons in consecutive pupal stages (P0-P5) of *Apis mellifera* and compared it with that of octopaminergic neurons in the adult brain. Octopamine was visualized using immunofluorescent staining and confocal laser scanning microscopy. Areas of particular interest were the neuropils of the olfactory pathway and somata clusters, which in a previous study (Kreissl et al., 1994) were found to be octopaminergic. Our results show that octopaminergic fibres can be detected in the calyces, the antennal lobes, the suboesophageal ganglion (SOG) and the lateral and medial antenno-cerebralis tract (lACT / mACT) as early as the P1 stage. Extrapolating from the distribution of octopamine-immunoreactive processes in the early pupal calyx, we found a compartmentalisation of this neuropil in the precursors of the lip- and collar-neuropils as early as P0. Although the processes innervating the lip-neuropils are known to also innervate the basal ring, this calycal subdivision was not clearly detectable until P5. The most conspicuous changes in the distribution of octopamine-immunoreactive fibres were found in the antennal lobes. Starting out as a wide meshed web spherically surrounding a largely octopamine-free center in P1, the investigated fibres develop during early pupal development to densely-arborized tufts in the bases of the olfactory glomeruli. A small group of octopaminergic somata are immunohistochemically educible at the second pupal stage (P1) in the medio-ventral SOG, known to be the location of the VUM-neurons (Hammer, 1993). During development

octopaminergic fibres extend into a second pair of medial tracts. These bear a strong resemblance to the VCBN-tracts described by Schröter (2002). These findings correspond to the growth of the octopaminergic somata cluster in the SOG and the increasing immunoreactivity in the ventral part of the α-lobes, the projection area of the VCBN-neurons. Beyond the octopaminergic somata clusters described by Kreissl et al. (1994) we found a new octopaminergic cell population ventral to the medial calyces of the adult brain. The primary neurites of these somata project into the protocerebral bridge and the ventral part of the central body, which exhibits considerable octopaminergic immunoreactivity. The apparent similarity in shape and position of the octopaminergic structures found in this study to the modulatory cells described earlier (Hammer, 1993; Schröter, 2002) leads us to make a suggestion regarding the identity of the described cells. This is another indication for the octopaminergicity of VUM and VCBN in the honeybee.

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The Virtual Brain Project: Comparison of expression patterns of different reporter genes driven by the same Gal4-enhancer trap line

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The expression patterns of Gal4-enhancer trap lines are highly dependent on the genomic positions of the used reporter constructs. In the two-component Gal4 enhancer trap system of Brand and Perrimon (Development 118: 401-415; 1993) tissue specificity is thought to be provided by the expression pattern of Gal4 in the driver line whereas the reporter construct is usually assumed to influence this pattern, if at all, only by the detectability and stability of the reporter. We have compared the expression patterns of the same driver / reporter combinations differing only in the genomic location of the reporter insert. For the driver line Gal4-201y different expression patterns were found in all (five) dual comparisons with three different reporter constructs. While the patterns agreed in several basic features they differed in many details which could not be explained assuming differences in general expression levels. Differences between reporter constructs were not larger than between genomic insertion sights of the same constructs. Variability among flies of the same genotype was much smaller. Data for other Gal4-driver lines will be presented.

Expression patterns are mapped into an advanced Standard Brain (Rein et al, 2002) in which all areas, including the so-called unstructured neuropil are labeled. For this purpose boundaries had to be draw between regions by convention. The newly defined neuropil areas are named according to the references below.

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692 3-D reconstruction of the beewolf brain, *Philanthus triangulum* F.

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Females of the European beewolf, *Philanthus triangulum* F. (Hymenoptera: Sphecidae) hunt for honeybees as larval food. They have to detect and identify honeybees and when flying back to the nest with the paralysed bee, females should be able to locate their burrow as fast as possible to avoid brood parasites. Males establish small scent marked territories near female nests and defend them against conspecific intruders for several days. Thus, females and males had to evolve complex sensory and neural capacities. In contrast to behavioral data, nothing is known about the brain structure of the beewolf,*Philanthus triangulum.* We investigated the brain structures of adult females and males and compared these with the well-studied brain of other hymenopteran wasps (Ehmer et. Al. , 2000, Smid et. , al 2001). Vibratome brain sections were treated with a monoclonal antibody against *Drosophila* synapsin, SYNORF1, (Klagges et al. ,1996) and neuropil was visualized with a CY-2 fluorescence probe. SYNORF1 acts as an synaptic neuropilmarker, binding to those brain regions that exhibit high synaptic densities, while somata regions remain unstained. Digital confocal scans were evaluated using 3D software from Amira (Indeed, TGS). The vibratome technique we used here allows high resolution confocal imaging of single brain structures. For example, subcompartments of the mushroom bodies (MBs), wich in *Philanthus*are highly differentiated, can be easily described according to the synaptic densities in its calycal and axonal parts. A crucial feature of the MB calyces, their presynaptic boutons, can be visualized and quantified which in further studies may be used as an structural parameter. In the antennal lobe of the male beewolf, which consists of approximately 130 glomeruli, two conspicious large glomeruli are located ventral and posterior adjacent to the dorsal lobe. In female species exists only one large glomerulus in a similar position. Macroglomerular structures as described in other parasitoid wasps in the medial part of the antennal lobe (Smid et. Al, 2001) are not found in either of both sexes of Philanthus triangulum. In the brain we find a subdivided β-lobe, a feature of the mushroom bodies also described in vespid wasps (Ehmer et al. 2000). Currently we undergo a detailed description of the brain of the specid wasp and compare it with other wasp groups and other Hymenoptera.

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Deficit of striatal calretinin-immunreactive GABAergic interneurons in a genetic animal model of primary paroxysmal dystonia 693

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The underlying mechanisms of various types of hereditary paroxysmal dyskinesias are still unknown but basal ganglia dysfunctions seem to play a critical role. In fact, recent immunohistochemical studies in the *dt*^{sz} hamster, a genetic animal model of a type of paroxysmal dystonia, have demonstrated a deficit of striatal parvalbumin-immunreactive (PV^+) GABAergic interneurons [1]. These data prompted us to investigate if the deficit of striatal interneurons is restricted to those which express parvalbumin. Therefore, in the present study we investigated the density of striatal calretinin-immunreactive (CR^+) interneurons, which represent a small population of striatal interneurons, positive for GABA, GAD_{67} and in part for choline acetyltransferase.

The cells were counted by using a stereological counting method in a blinded fashion. A significant reduction in the density (-20%, $p<0.001$) of calretinin-immunreactive interneurons was found in *dt*^{sz} mutants in comparison to age- and gender-matched non-dystonic control hamsters. These results indicate that the deficit of striatal interneurons is not restricted to those which are immunreactive for parvalbumin. Since the striatal calretinin-immunreactive interneurons are GABAergic like the parvalbumin-immunreactive ones, the inborn deficit of striatal GABAergic interneurons in *dt*^{sz} hamsters implies that the GABAergic system deserves attention in human dyskinesias, particularly in types in which GABA-potentiating drugs exert beneficial effects. Striatal dopaminergic overactivity in *dt*^{sz} mutants, indicated by previous pharmacological, neurochemical and microdialysis studies, may be important for the manifestation of dystonic attacks but is probably secondary to the reduced striatal GABAergic inhibition.

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Reduction of striatal nitric oxide synthase-immunreactive interneurons in an animal model of primary paroxysmal dystonia 694

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Primary paroxysmal dystonia is a common movement disorder, whose pathophysiology is poorly understood. Recent studies have shown a significant reduction of striatal parvalbumin expressing GABAergic interneurons (PV^+) in *dt*^{sz} hamsters, a genetic animal model of this disease. To examine if PV^+ interneurons are the only affected type of striatal interneurons, in the present study, we investigated the density of nitric oxide synthase expressing interneurons (NOS^+). Neurons, characterised by containing NOS,

somatostatin, neuropeptid Y, substance P and GABA, constitute only 1-2% of striatal cells.

The striatal density of NOS^+ interneurons was determined in *dt*^{sz} hamsters and age- and gender-matched non-dystonic control hamsters by using a stereological counting method in a blinded fashion. We found a significantly reduced density (-21%, $p<0.001$) within the striatum of mutant hamsters in comparison to control animals, particularly within the dorsomedial (-38%, $p<0.001$), dorsolateral (-30%, $p<0.001$) and the ventrolateral (-17%, $p=0.037$) regions of the striatum.

These results suggest that not only a deficit of PV^+ interneurons but also a decreased density of NOS^+ interneurons could contribute to an abnormal innervation of basal ganglia output structures as shown by previous electrophysiological studies in mutant hamsters. Further investigations are needed to clarify the special role of a NOS^+ interneuron deficit in the pathophysiology of this movement disorder.

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695 Analysis of cell numbers in immunohistochemically stained brain sections using a computerized image analysis system

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Neuronal networks are established by cells with different characteristics, for example the ability to produce specific transmitters or to buffer calcium-ions by expressing calcium-binding proteins. To understand neuronal networks, it is essential to know, how many cells express the specific markers, which can be visualized immunohistochemically. Cells numbers then have to be evaluated.

Here we propose a reliable method for automatic counting of cells in 5 µm paraffin sections of the brain, labelled with different antibodies (against NeuN, parvalbumin, GABA and GAD) or stained with Nissl. Images of stained sections are acquired with a CCD-camera on the microscope and are converted to binary images by thresholding. Clusters of “ON pixels” (value of 1) corresponding to cell bodies are selected based on size. The parameters of the algorithm (intensity range and cluster-size) are adjusted for different methods of staining according to expert knowledge. Cell size is simultaneously determined by the histogram.

The presented automatic cell counting method (ACCM) provides correct counting results as demonstrated by a comparison of computational results with counts gained by human experimenters and with a commercially available image analysis system. On the basis of ACCM counts small and perhaps physiologically relevant differences in the number of labelled cells can be revealed, as demonstrated here for the GABAergic system after two hours of an electrical stimulation, changing neuronal network characteristics.

The program can be downloaded for free as an IDL-version under https://www.neurop.ruhr-uni-bochum.de/~alia/ACCM

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Actin is highly expressed in the honeybee brain neuropiles 696

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Over the last decades it has been discovered that in mammalian brain actin is intimately involved in processes related to synaptic plasticity. In this study, we demonstrate that F-actin is highly expressed in the brain of honeybee. F-actin was labelled with phalloidin-rhodamine on 40 mm thick vibratome sections. The higher associative brain centres ? mushroom bodies (MBs) and antennal lobes - showed remarkable fluorescence with the highest level in the MB calyces. Within the latter, the fluorescence exhibited a pattern characterized by small spherical particles. The observed pattern might reflect microglomerular structure of the MB calyces. In antennal lobes, glomeruli showed significant fluorescence, but, unlike that in MB calyces, it was homogeneous. In contrast to the neuropiles, the regions occupied by neuron somata did not show fluorescence. The elevated level of actin in the honeybee brain neuropiles may indicate the similarity in the mechanisms of synaptic plasticity in vertebrates and insects.

In order to visualize actin at ultrastructural level, MB calyces were examined using conventional transmission electron microscopy. Surprisingly, within the neuropile, neither presynaptic boutons nor postsynaptic spiny endings exhibited well developed filamentous cytoskeleton. Examination of profiles belonging to antennal lobe projection neuron (sensory input), labelled intracellularly, and profiles belonging to GABA-immunoreactive neurons (inhibitory input) also failed to demonstrate microfilamets in their cytoplasm. This result might be explained by destabilization of actin filaments during sample preparation for standard electron microscopy (Dino and Mugnaini, 2000). Thus a special treatment of samples combined with the specific labelling of actin for electron microscopy are needed to study subcellular distribution of actin within the neuropile.

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697 Localization of the neuropeptide angiotensin II and its reaction sites involved in the circadian control of blood pressure in normotensive and transgenic-hypertensive rats at three zeitgeber times

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Variations of the circadian rhythms in blood pressure [BP] and heart rate [HR] have been described in various strains of normo- and hypertensive rats using telemetry. In normotensive and spontaneous-hypertensive rats, BP and HR levels are higher during the activity period at night. However, in transgenic-hypertensive rats [TGR], bearing a mouse renin gene, a dissociation between rhythms in BP and those in HR and locomotor activity were described with peak values in BP during the diurnal resting phase.

All these rhythmic patterns can be abolished by lesioning the suprachiasmatic nucleus [SCN]. An analysis of the hypothalamic origin and target areas of enzymes regulating and modulating blood pressure should help to further understand underlying processes.

By means of immunohistology on the light- and electron microscopic level we studied the subcellular distribution of two component parts of the circulatory control system: Angiotensin II [ANG II] and its transmembrane receptor [AT1]. We compared results from normotensive Sprague-Dawley rats [SDR] and transgenic-hypertensive rats [TGR], both groups 10 weeks old, which were sacrificed at different times of day.

The poster shows:

-(a) comparative anti-AT1-immunoreactivity of the SCN and the periventricular nucleus (PVN) in rat brains fixed at different times of day, and (1) strong membrane stainings on finest capillary endings of the organum vasculosum lamina terminalis [OVLT]. (2) clear AT1-receptor immunosignals in the cytoplasm of neurons of the median preoptic nucleus [MnPO] and at the membranes of their axonal processes. (3) AT1-immunoreactive nervous terminals and non-proliferating cells of the choroid plexus [ChP].

-(b) anti-AT1-immunoreactivity on the light and electron microscopic level. As a prerequisite for this part of our study, we developed a fast reproducible DAB-staining technique for glutaraldehyde- and paraformaldehyde-fixed brain tissue. It allows a reliable visualization of immunoreactivity on both microscopical level and might also provide an alternative to colloidal gold-labelling techniques which do not deliver clear light

microscopic images. We demonstrate corresponding ultrastructural localization of ANG II and its transmembrane receptor AT1 at two sites of neuropeptide transport: (1) in the perivascular region of brain parenchyma vessels and (2) at the ependyma of the third ventricle.

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Localization of methionine sulfoxide reductase A (MSRA) in the mouse brain 698

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The lifespan of organisms is influenced by both genetic and environmental factors. Biological oxidants, such as hydrogen peroxide, hydroxyl radicals, hypochlorite and peroxynitrite, are postulated to accelerate the aging process. Furthermore, they may contribute to the development of neurodegenerative disorders, such as Parkinson's and Alzheimer's disease. Methionine residues in proteins are readily oxidized to methionine sulfoxide (Met-R-O, and Met-S-O) by various reactive oxygen species (ROS). In contrast to other oxidized amino acids, MetO can be reduced enzymatically to Met by methionine sulfoxide reductases (MSRs). MSRAs stereospecifically reduce Met-S-O while MSRBs reduce Met-R-O. Overexpression of MSRA in neuronal tissues markedly increased the lifespan of *Drosophila melanogaster*, corroborating the importance of ROS and MSR activity in aging, especially in the nervous system (Ruan et al., 2002; PNAS 99, 2748-53). In addition to their roles in oxidative damage repair and ROS scavenging, MSRs may have regulatory roles (Hoshi & Heinemann, 2001; J. Physiol. 531, 1-11).

In this study we determined the localization of MSRA, an enzyme that reduces Met-S-O, in the mouse brain using antibodies raised against recombinant human MSRA in rabbits. The specificity of the antibodies was confirmed by Western blot analysis of mouse brain extracts, in which a single band at 23 kDa was observed. Furthermore, methionine sulfoxide reductase A-like immunoreactivity (MSRALI) could be blocked by preincubation of the antibodies with the recombinant hMSRA protein. 50-µm sections of brains from intracardially perfused mice were incubated free-floating with antibodies. MSRALI occurred virtually in all parts of the brain, but the expression pattern of MSRA varied considerably. The strongest immunoreactivity was detected in neurons of the mesencephalic trigeminal nucleus (MTN), which is a part of the trigeminal sensory system

(TSS). Most neurons of the TSS are localized in the trigeminal ganglion in which the majority of the neurons also revealed a strong immunoreactivity. Furthermore, we found notable MSRALI in about 60% of all dorsal root ganglion neurons (DRG-neurons). In neurons, only the cell somata and dendrites showed immunostaining. In the olfactory bulb only mitral cells exhibited a moderate MSRALI. The stained dendrites of these second-order neurons form synaptic contacts with the unstained axons of the olfactory sensory neurons in the glomeruli. Furthermore, a large number of pyramidal cells (level III and V) with moderate to strong MSRALI were visible in the cerebral cortex. These pyramidal cells were exclusively located in the sensory cerebral cortex, with highest intensity in the cingulate cortex. In addition to olfactory bulb and cerebral cortex, appreciable immunoreactivity was detected in the Purkinje cells of the cerebellum, in neurons of the basal ganglia, thalamic nuclei, pontine reticular nucleus and in hippocampal CA1 pyramidal neurons.

The remarkable differences in the MSRALI observed in this study may reflect the metabolic activity of the corresponding cells and/or a function of MSRA in specific neuronal processes.

699 Calcium-binding proteins in the cerebellum of the japanese quail

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Previous comparative investigations revealed substantial differences regarding the distribution patterns of calcium-binding proteins (CaBPs) in parts of the quail´s brain, especially in structures related to visually guided behaviour. Parts of the cerebellum are also involved in the control of this reaction, but its chemoarchitecture remains to be elucidated. Like that of other amniotes, the avian cerebellum may be divided into a more superficial cortical region and a group of deep cerebellar nuclei. Divisions of the avian cerebellar cortex are the granular, molecular, and Purkinje cell layers, whereby the organization of its neuronal elements is comparable to that of mammals with granular and Golgi cells in the granular layer, the molecular layer with basket and stellate cells, and the Purkinje cells. Known subdivisions of deep cerebellar nuclei are the nucleus cerebellaris internus, nucleus cerebellaris internus, pars ventromedialis (vm), the nucleus cerebellaris intermedius with its internal, intermediate, and intercalate parts, the processus lateralis cerebello-vestibularis, the nucleus cerebellaris lateralis (CbL), and the nucleus infracerebellaris.

Single immunoperoxidase labelling was used to study the distribution of parvalbumin (PARV), calbindin-D28k (CB), calretinin (CR), and S100A1 in the cerebellum of the Japanese quail (*Coturnix coturnix japonica*). In addition, double and triple immunofluorescence labelling were applied to investigate the degree of cells co-expressing PARV, CB, and CR.

The mapping of CaBPs in the cerebellar cortex revealed their different distribution patterns in all layers. PARV-immunoreactivity (ir) was detected in the neuropil (np) of the molecular and granular layers, whereas the staining of somata remained restricted to

Purkinje cells - and with less intensity - to basket and stellate cells. Fibres in the white matter were also PARV-immunopositive. CB-ir has been mapped in all cells of the cerebellar cortex and was most striking in the Purkinje cells and in basket cells of the molecular layer. The fibres of the molecular layer are densely marked. CR-ir was observed in fibres and cells of the granular, molecular layers, and white matter, but was not detectable in Purkinje cells. The distribution pattern of S100A1-ir resembled that of PARV-ir, but was less intense. In addition, strong S100A1-ir was detected in astrocytes within the molecular layer.

The labelling of CaBPs in cells and fibres of the cerebellar nuclei was similar in nearly all structures. Only the CbL (CR-ir in fibres and np, but absent in perikarya) and the vm (CR- and CB-ir in cells, but PARV- and S100A1-ir in the np) were differently marked. Taken together, there are the same distribution patterns of CaBPs in all foliae of the cerebellar cortex, but distinct cell types displayed a different content of CaBPs in perikarya and fibres, whereas in the deep nuclei these differences concern only CbL and vm. Finally it is noteworthy, that the distribution of CaBPs show a remarkable degree of co-expression with GABA in all cerebellar parts.

Comparative analysis of NADPH-diaphorase staining in the brain of the moth *Manduca sexta* and the locust *Schistocerca gregaria* 700

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The enzyme nitric oxide synthase (NOS) generates the gaseous signalling molecule nitric oxide (NO), which plays an important role in the nervous systems of vertebrates and invertebrates. In locusts, histochemical staining for NADPH-diaphorase (NADPHd) is a reliable marker for NOS-containing neurons; moreover, its sensitivity is dramatically improved by fixation in methanol/formalin (Ott et al., 2002, J Comp Neurol 448: 165). Here we used this technique to map putative NO-producing neurons in the brain of *M. sexta* in comparison to the well-studied brain of the locust.

Both species showed extensive, distinct, and intense NADPHd staining. The antennal lobes displayed prominent NADPHd activity that derived from interneurons, whereas antennal nerves were unstained. In *M. sexta*, strongly stained local neurons accounted for most of the NADPHd in the lobes, but a group of projection neurons with intensely labelled axons in the outer antenno-cerebral tract was also present. This contrasts with the situation in the locust, where NADPHd was restricted to the local neurons described in Elphick et al. (1995, J Exp Biol 198: 821) and where no antenno-cerebral connections were stained.

In the median protocerebrum of both species, the most prominent staining was seen in the mushroom bodies, followed by the central complex. The protocerebral lobes surrounding these two neuropils were invaded by loose meshworks of NADPHd-reactive fibers from a variety of neurons. In the mushroom bodies of *M. sexta*, the staining was

clearly associated with subpopulations of intrinsic Kenyon cells. This is in striking parallel to the locust, where strong NADPHd in the mushroom bodies, previously attributed to extrinsic neurons (e.g., O'Shea et al., 1998, Neuroreport 9: 333), has recently been demonstrated to reside in subsets of Kenyon cells (Ott et al., 2002, FENS Abstr vol 1, A185.19). In the central complex, strong staining was found in the central body and in the protocerebral bridge of both species. However, in *M. sexta* only the upper division of the central body exhibited NADPHd activity, while the upper and lower division were stained in *S. gregaria*.

Contrasting with these similarties in the brain, the NADPHd staining pattern in the optic lobes was strikingly different between both species. In *S. gregaria*, intense NADPHd was widespread with particularly strong activity in lamina monopolar cells and the lobula complex, confirming the analysis of Elphick et al. (1996, J Exp Biol 199: 2395). In contrast, only sparse staining occurred in the optic lobes of *M. sexta*.

In conclusion the NADPHd staining pattern in the brains of both insects showed striking similarities, particularly in the antennal lobes and mushroom body, but marked differences in the optic lobes. The results suggest that NO, as in the locust, serves a prominent role in the olfactory system of *M. sexta* as well as in several higher-order brain areas.

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701 Fine structural immunocytochemistry: A manner of multiple labeling on an invertebrate neurosecretory system

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Ultrastructural localization of a biochemically defined antigen using immunogold reagents is a suitable method to localize it distinctively inside or outside a single biological compartment or attached to a membrane region and render it easily quantifiable. If one succeeds in keeping the antigenicity at a detectable level and maintaining cell structure satisfactorily, problem of reaction product diffusion will also be avoided.

Double labeling could be carried out on the two sides of the grid to avoid cross-reaction. For multiple labeling difficulties may come when antisera employed were raised in the same host as primary antiserum molecules could have several open binding sites remained after completion of the first reaction. To solve this problem adaptation of monovalent Fab fragment molecules is suggested. Fab fragments with covering surface remained accessible on the first primary eliminate cross reactions with secondary serum of the subsequent reaction. Third process could be carried out on backside of the grid and to differentiation of the single antigenes in the three reactions antisera conjugated with gold particles of different size were used.

Compared to light microscopic versions of the procedure presented earlier – because of the easy quantification of immunogold labelings - special care should be taken to find appropriate concentrations enough to reach sufficient strength and keep reduced back-

ground. Further several combinations of control experiments are needed to exclude false reactions and artefacts.

Cockroach abdominal perisympathetic organs are composed of several branched axons coming from a limited number of abdominal median neurosecretory cells. Light microscopic immunoreactions proved the presence of six recently identified invertebrate neuropeptides in these structures. Fab method was applied to study occurrence of these peptides at the ultrastructural level. Our protocol was suitable to reveal whether these peptides are co-exist in the same cell but in different granules or co-stored to the same vesicle.

Evolution of serotonin-immunoreactive neurons in the arthropod ventral nerve cord 702

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Comparative studies on structure and development of the nervous system contribute important arguments in the debate on the phylogenetic relationships within the Arthropoda. In the present report, the arrangement of serotonergic neurons was examined in the ventral nerve cord of representatives of the Branchiopoda, Malacostraca, Chelicerata, Chilopoda and Diplopoda and compared to that in Hexapoda. The results indicate that these neurons in Branchiopoda and Malacostraca have corresponding counterparts in the Hexapoda, hence representing a plesiomorphic character of Crustacea and Hexapoda. Based on the finding of these homologies, the arrangement of serotonergic neurons in a model trunk ganglion of the mandibulate ground pattern was reconstructed as comprising an anterior and a posterior pair of serotonergic neurons per hemiganglion, each cell with both, an ipsilateral and a contralateral neurite. Starting from this ground pattern, the evolutionary diversification of this class of neurons within the mandibulate groups is discussed. Whereas members of the Chilopoda partly fit into this proposed ground pattern, the Diplopoda as well as the Chelicerata clearly show different patterns of serotonergic neurons. The impact of these data on the position of the latter three taxa within the conflicting hypotheses on arthropod relationships is outlined.

The role of the calcium sensor protein VILIP-1 in neuronal signalling 703

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Calcium plays a key role as second messenger in neuronal signalling processes,thus, influencing important cellular processes i.e. neuronal plasticity but also pathophysiological processes of the CNS. EF-hand calcium-binding proteins have an important role as mediators of calcium signals. The EF-hand super-family comprises a

family of intracellular neuronal calcium sensor (NCS) proteins including the Visinin-like proteins (VILIP-1 to -3) the investigation of which may gain new insights into the specific role of calcium signalling in the CNS.

We have recently explored whether the calcium-dependent membrane localization of VILIPs, the so called calcium-myristoyl switch, can serve as a cellular signalling mechanism in living cells. The translocation of myristoylated and non-myristoylated green fluorescence protein-tagged NCS proteins to subcellular membrane structures was examined in transfected cell lines and primary hippocampal neurons. The stimuli-induced and reversible translocation of the GFP-fusion proteins of VILIP-1 and -3 to cellular signalling compartments, such as the Golgi network or membrane specializations in neuronal dendrites, was monitored by fluorescence and time-lapse microscopy. Our data raise the possibility that the calcium-myristoyl switch of VILIP-1 and -3 provides a fast signalling mechanism to shuttle cellular signals and signalling molecules to cellular compartments and/or to influence membrane-associated signalling effectors in a calcium-dependent manner. In line with this notion we have found functional influence of VILIP-1 on different neuronal signalling cascades. In transfected cell lines an effect of VILIP-1 expression on cAMP- and cGMP-signalling, most likely via interaction with cyclase enzymes has been observed and has been confirmed in cerebellar and hippocampal neurons. Moreover, VILIP-1 is also able to functionally affect neurotransmitter receptors, such as nicotinic acetylcholine receptor.

Thus, our results indicate that a main function of the calcium sensor protein VILIP-1 may be the calcium-dependent modulation of neuronal signalling cascades, including cyclic nucleotide-, receptor and ion channel signalling

704 Inhibition of Rat Brain Mitochondrial Respiratory Chain Enzymes by Dopamine

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Dopamine has been shown to cause mitochondrial swelling, a decrease in state 3 respiration and an inhibition of complex I activity during *in vitro* incubation and the phenomena have been implicated in the death of dopaminergic neurons in Parkinson's disease.In the present study, we re-examined the effects of dopamine on mitochondrial respiratory chain enzymes.Dopamine(0.1-0.4mM) caused a dose-dependent inhibition of both complex I and complex IV activities during *in vitro* incubation of rat brain mitochondria.The inhibition was slow but progressive at pH 7.4 and after 2h of incubation the activities of complex I and complex IV were reduced by more than thirty-five and fifty percent respectively by dopamine(0.4mM). The enzyme inhibition was apparently mediated by dopamine oxidation products and only some direct effect of dopamine on complex I and not on complex IV could be detected.Oxygen free-radicals, the toxic by-products of dopamine oxidation , were also not involved in the inhibition process,since the latter phenomenon was neither prevented by the radical-scavengers like mannitol(20mM) or dimethyl sulphoxide (20mM) ,nor by the anti-oxidant enzyme catalase.On the otherhand,reduced glutathione(5mM) or cysteine (5mM) which forms adducts with

quinones completely blocked the effects of dopamine on complex I and complex IV, suggesting a role for reactive quinones derived from dopamine oxidation in this process.These *in vitro* data are relevant in the context of mitochondrial dysfunction reported in Parkinson's disease.

Binding partners for acetylcholinesterase in the mammalian CNS

705

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The classical function of acetylcholinesterase (AChE) is to hydrolyze the neurotransmitter acetylcholine at the cholinergic synapses. However, the presence and also the function of AChE in tissues that are not innervated directly by cholinergic nerves remains unclear. There is evidence of non-classical actions of AChE, showing a remarkable diversity of functions, e.g. promoting neuritogenesis, cell adhesion, synaptogenesis, activation of dopamine neurons, amyloid fiber assembly, but the actual role of AChE in these phenomena remains to be tested in a more direct manner.

We are interested to elucidate the mechanism through which acetylcholinesterase exerts its adhesive and morphogenic functions. The high degree of sequence homology between AChE and the family of synaptic cell adhesion proteins neuroligins, suggest that AChE may act as a signaling or cell adhesion molecule during neuritogenesis and synaptogenesis. AChE is an extracellular enzyme, and doesnt have a transmembrane and intracellular domain, therefore is not able to transduce signals into the cell .

The solution for this problem may rely in the presence of unknown binding partners for AChE, that should have the following requisites: be expressed in the same tissues and at the same developmental stages as AChE, have both an extracellular and an intracellular domain connected through one or more transmembrane domains, and possibly be able to initiate an intracellular signaling cascade.

We are now looking for binding partners by using the dimeric 19-3 form of AChE immobilized on a column through which we are running mouse brain extracts. The chormatography is a good enrichment step, and the eluate is further used for surface plasmon resonance experiments. Using cholinesterase bound to a CM5 chip and a BIACORE microrecovery program, we were able to retrieve possible AChE binding partners. The BIACORE recovered volume is separated by SDS-PAGE and the gel stained using the silver staining. The results show the presence of more binding partners of different molecular weights. We are currently working on optimizing the composition of buffers and on identifying these proteins.

706 Impairing mitochondrial metabolism in hypoglossal motoneurons from mouse: Implications for amyotrophic lateral sclerosis (ALS)

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Mitochondrial dysfunction has been implicated in the pathogenesis of amyotrophic lateral sclerosis (ALS), a neurodegenerative disease characterized by selective motoneuron loss. A yet undetermined question is, how impaired mitochondrial metabolism contributes to motoneuron degeneration. In the present report we therefore investigated the impact of impaired mitochondrial metabolism on neuronal excitability and $[Ca^{2+}]_i$ in motoneurons. Inhibition of the mitochondrial respiratory chain using cyanide (CN) increased motoroutput recorded from hypoglossal (XII) rootlets, suggesting an enhanced activity of hypoglossal motoneurons (HMs) during CN action. Consistently, on the cellular level, CN depolarized HMs by 8.9 ± 1.0 mV and increased spontaneous action potential activity. Simultaneous measurement of $[Ca^{2+}]_i$ using fura-2 showed that CN increased

resting $[Ca^{2+}]_i$ i) due to occurrence of action potentials and ii) due to release of Ca^{2+} from mitochondria. Depolarization of mitochondria by CN also disturbed cytosolic Ca^{2+} clearance as indicated by 2-fold slower recovery of activity-dependent Ca^{2+} transients. Regarding the low cytosolic Ca^{2+} buffering capacity of hypoglossal motoneurons, these events may contribute to degeneration of motoneurons in ALS i.e. by sensitizing them to excitotoxic cascades.

707 Phosphodiesterase expression and second messenger levels in two human glioblastoma cell lines

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Many tumour cells exhibit low levels of cAMP as a consequence of over-expression of cyclic nucleotide phosphodiesterases (PDEs) of the subfamily 4. Manipulating intracellular cAMP levels by inhibition of tumour-associated PDEs appears to be a promising tool in tumour growth inhibition. In five of six highly malignant human glioblastoma cell lines originating from the 60 tumour cell line panel of the NCI, Ca^{2+}/calmodulin (CaM) -sensitive PDE1C was the predominant enzyme family, whereas rolipram-sensitive PDE4 activity was minor. PDE1C and PDE4 have affinities for cAMP in the low µmolar range, therefore we speculated that PDE1C might play a pivotal role in

cAMP homeostasis of CNS tumour cells in a similar fashion as we have described previously for PDE4 in epithelial tumour cells.

Two glioblastoma cell lines were studied in more detail. High PDE1C activity, stimulated about three-fold by CaM, was expressed in SNB75, whereas PDE4 activity was low. In contrast, SF295 showed no CaM-sensitive PDE1C activity, but relatively high PDE4 activity. Semi-quantitative Real Time RT-PCR analysis demonstrated a similar PDE expression pattern at the transcription level.

Cytosolic cAMP content was three times lower in SNB75 than in SF295, whereas the intracellular Ca^{2+} level in SNB75 was significantly higher than in SF295. Application of 10mM cyclopiazonic acid (CPA) concomitant with or without 2mM Ca^{2+} showed that SNB75 displays a higher Ca^{2+} storing capacity, a faster Ca^{2+} release from stores and a more effective capacitive calcium entry than SF295 cells.

These data suggest that in SNB75, the large intracellular Ca^{2+} pool causes activation of calmodulin, leading, together with an over-expression of PDE1C, to increased CaM-sensitive PDE1C activity, thus resulting in a low cAMP level. In SF295 cells however, despite high PDE4 activity, high cAMP levels were observed. The two cell lines investigated, SNB75 and SF295, offer a useful model for further studies on the relationship between PDE1/4 expression and second messenger levels in CNS tumour cells.

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The CNS-proteoglycan brevican is located in perineuronal nets in primary hippocampal cultures

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Brevican is a neural-specific proteoglycan of the brain extracellular matrix (ECM) which is specifically abundant in the terminally differentiated CNS. It is expressed by neuronal and glial cells and as a component of the perineuronal nets (PNN) it decorates the surface of large neuronal somata and primary dendrites. PNN are thought to be involved in synapse stabilization and insulation, ion buffering, perisynaptic concentration of growth factors and linking the extracellular with intracellular matrix. Interestingly, these nets can also form in mature primary neuronal cultures. To understand the role and development of PNN better, we investigate the brevican immunorectivity in 1, 2 and 3 weeks old primary hippocampal cultures. Whereas brevican staining is very weak after 7 DIV, immunoreactivity steadily increases in the second and third week. Double labelings with synaptic markers suggest that brevican-containing PNN embed some synapses. The association with synapses is confirmed by biochemical fractionation studies, showing both the major secreted isoform and the GPI-anchored minor isoform to be enriched in synaptic protein fractions. Both isoforms are generally found to be associated with cellular membranes and the membrane association is in part sensitive to chondroitinase ABC and hyaluronidase treatment. This could provide a mechanism how PNN are anchored on neuronal surfaces. Funded by the DFG (SE 952/2-2, 2-4).

Related literature:
Seidenbecher C.I., Smalla K.-H., Fischer N., Gundelfinger E.D. and Kreutz M.R. 2002. Brevican isoforms associate with neural membranes. J Neurochem 83: 738-746.

709 Divalent Iron Accelerates $A\beta_{1-40}$-Dependent Signal Transduction Cascades and Toxicity in Neuronal Cells

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Losses of neuronal cells in Alzheimer's disease (AD) patients have been attributed in part to oxidative stress (OS) that may be amplified by divalent metal ions. Fe^{2+} has been shown to enhance free radicals production and also promote Aβ radicalization. There is presently very little information as to the mechanism by which metal ions such as iron modulate cellular signaling cascades either via an Aβ-dependent or independent action. To examine that, dissociated rat cerebral cultures were treated either separately or with a combination of Fe^{2+} (5 μm) and the $A\beta_{1-40}$ (5 μm) and several OS parameters including free radicals formation and lipid peroxidation respectively, were measured by dichlorofuorescein staining and thiobarbituric acid assays. Additional parameters included mitochondria function, caspase activation and TUNEL staining (Kuperstein and Yavin, Eur J Neurosci, Vol. 16: 44-54, 2002). A combination of Fe^{2+} and $A\beta_{1-40}$ caused delayed ERK activation, enhanced p38 MAPK and caspase activity ultimately resulting in cell death. It also caused a reduction in Akt activity and Ser-136 BAD phosphorylation as well as a decrease in several PKC isoforms. In contrast, addition of $A\beta_{1-40}$ without iron enhanced PKC levels, stimulated Akt and enhanced Ser-136 BAD phosphorylation suggesting a potential anti-apoptotic function of the peptide. A number of inhibitors including GF, a PKC inhibitor, wortmaninn and LY, both blockers of the ΠI3K/Akt pathway enhanced $A\beta_{1-40}Fe^{2+}$ -induced toxicity, while U0126, a MEK inhibitor and SB, a p38 MAPK inhibitor, prevented cell damage and apoptosis. The oxidative stress component of the $A\beta_{1-40}Fe^{2+}$ induced toxicity could be attenuated by NAC and catalase antioxidants, by the iron chelator deferroxamine and by a pentameric $A\beta_{1-40}$ analog known to be an $A\beta_{1-40}$ aggregation inhibitor. These findings further support the possibility that metal ion chelation, antioxidants and pharmacological inhibition of pro-apoptotic kinase cascades may be beneficial in reducing the severity of Alzheimer's disease. Supported by a grant from the Gulton Foundation.

710 Cholesterol homeostasis in neurons

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Our recent study (Mauch et al., 2001 Science 294: 1354-1357) showed that glia-derived cholesterol promotes synapse formation in cultures of purified rat retinal ganglion cells (RGC). This provokes the hypothesis that neurons depend on glia-derived cholesterol.

So far, surprisingly little is known about cholesterol homeostasis in neurons. To address the question whether neurons synthesize cholesterol, we used radioactive labeling in combination with thin-layer chromatography to detect new synthesized cholesterol. Our results show that neurons from postnatal brain produce cholesterol when cultured under defined, glia-free conditions. Currently, we are investigating whether factors from glial cells regulate this process. The assumption that neurons import cholesterol from glia postulates that these cells like all other cells in the body possess means to protect themselves from sterol overload. A first hint came from our study on glia-induced changes in the protein-pattern of purified RGCs. Two-dimensional gel-electrophoresis and protein-identification by mass-spectrometry indicated the presence of apolipoprotein A1 (ApoA1) in neuronal membrane-fractions. Using RT-PCR and suitable primers, we detected mRNA encoding ApoA1 in purified RGCs suggesting that - in contrast to current opinion - ApoA1 is produced by neurons. Subsequent gel electrophoretic separation and western-blot analysis of proteins contained in neuron-conditioned medium showed that RGCs secrete ApoA1 into the extracellular space. A combination of non-denaturating gradient gels and western blots revealed that secreted apoA-1 is contained in large particles ranging from 250 to 700 kD in size. Considering the well-established fact that outside the brain ApoA1-containing high-density lipoproteins mediate the reverse cholesterol transport from peripheral organs like muscles back to the liver, we are testing now the hypothesis that neurons employ a similar mechanism to get rid of excess cholesterol.

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Long-term depression of presynaptic release from the readily-releasable vesicle pool induced by NMDA receptor-dependent retrograde NO

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Postsynaptic changes are presently believed to fully account for NMDA receptor dependent synaptic long term depression (LTD) and potentiation (LTP). Using dual photon laser scan microscopy of FM1-43 to directly visualize presynaptic vesicular release at Schaffer collateral-CA1 excitatory synapses in hippocampal slices, we demonstrate reduced release associated with LTD, and enhanced release during LTP. Selective loading by hypertonic shock of the readily-releasable vesicle pool (RRP) showed that both LTD and LTP of release were due to selective modifications of release from the RRP. LTD and LTP of RRP release both required NMDA receptor activation and production and extracellular diffusion of the intercellular messenger NO and activation of cyclicGMP-dependent protein kinase. These data demonstrate the ability of NMDA receptor-activated synaptic plasticity to elicit bidirectional, long-term changes in release from the RRP in intact hippocampal slices.

712 Plasticity of mushroom body-extrinsic neurons in the honeybee brain

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We recorded intracellularly from mushroom body-extrinsic neurons at the α-exit. Short electric pulses were used to stimulate the presynaptic neurons, the Kenyon cells, at their input sites (the calyx region). One of the neurons frequently recorded in this region is the PE-l neuron, but other neurons responding to olfactory and/or visual stimulation were also recorded. Many of the neurons were intracellularly marked. The electric stimulus was set to an intensity that reliably elicited one or two spikes in the recorded neuron. Double pulse facilitation and tetanic stimulation with different protocols were applied either alone or in combination with depolarization of the recorded neuron.

Double pulse facilitation or depression lasting for short periods were found frequently in different types of neurons. Facilitation or depression was not related to neuron type. In particular, the PE1 neuron may respond with double puls facilitation or inhibition. The resons for these differences are not known. Tetanic stimulation (100 Hz, 1 sec) induced either short-term potentiation or depression in various types of neurons. Again the neuron type appears not to determin whether short-term potetiation or depression is induced. Long-lasting potentiation (LTP) was induced only when the neuron was depolarized during tetanic stimulation, and only in the PE 1 neuron. In 6 cases the PE 1 could be recorded for more than 40 mins. Excitability was high during the entire recording time.

This is the first demonstration of LTP in a neuron of the insect brain. Our results indicate that the output synapses of the mushroom body neurons are potential sites of associative learning in addition to their input sites (the calyces), for which associative plasticity has been demonstrated. We conclude that mushroom body-extrinsic neurons at the α-exit are highly plastic. It is not clear what determines the short lasting plasticities in these neurons. We speculate that the modulatory status of the animal regulates the form of short-term synaptic plasticity. Long-lasting plasticity, however, was found only in the form of associative LTP and only in a particular neuron, the PE.

Combining intracellular recording with intracellular marking allowed us to identify the particular neuron. Several neuron types were found and classified. In the case of the PE1 neuron we demonstrated that this neuron receives input across the peduncle of the mushroom body, making it unlikely that it receives selective input from a subset of Kenyon cells (e.g. the so-called γ-Kenyon cells).

NMDA receptor mediated postsynaptic responses are reduced in neocortical neurons from α-neurexin deficient mice 713

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Neurexins are highly polymorph, presynaptically localized cell surface proteins that bind to postsynaptic cell adhesion molecules and intracellular PDZ-domain proteins. Using mutant mice lacking all α-neurexins (triple KO) we have recently found that they function as important regulators of Ca^{2+}-dependent neurotransmission, which is apparently based on an impaired function of voltage-gated Ca^{2+} -channels. We have now studied, whether postsynaptic responses mediated by ionotroptic glutamate receptors are also affected in α-neurexin deficient mice.

Triple KO mice die shortly after birth. Therefore, we studied postsynaptic responses in whole-cell patch clamp recordings at glutamatergic synapses in neocortical slices obtained from newborn triple KO and control mice and cultured for 25 to 40 DIV. Evoked AMPA and NMDA PSCs were pharmacologically isolated and recorded at holding potentials of ?80mV and +40mV. Miniature PSCs were examined at a holding potential of ?80mV under Mg^{2+} free conditions in the presence of picrotoxin and tetrodotoxin. We observed (1) a more than 50 % reduction of the amplitude ratio of evoked NMDA and AMPA PSCs in triple KO compared to control mice. These findings were confirmed with recordings of mPSCs (2) showing a 50% reduction in the NMDA receptor mediated component. Interestingly, experiments on dissociated, GFP-expressing wildtype neurons after integration into cultured slices from triple KO mice revealed that (3) the reduction in the NMDA component depends on the expression of α-neurexins in the postsynaptic neuron itself, and thus (4) appears to be independent of the properties of the neocortical network, e.g. the activity level of the surrounding neurons.

Functional synaptic integration of mouse ES cell-derived neurons in neocortical networks 714

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The analysis of functional synaptic integration of stem cell-derived neurons into the highly complex neocortical network is a crucial prerequisite for developing stem cell-based therapeutic approaches for neurodegenerative diseases of the neocortex. We are studying the functional synaptic integration of mouse ES cell-derived neurons into neocortical networks with particular emphasis on long-term synaptic plasticity as a cellular mechanism of learning and memory.

Two different culture systems are used for electrophysiological analysis with patch clamp recording. First, a simple, easily accessible modell system for synaptic integration has been developed. This modell system consists of primary cultured, neocortical explants from embryonic mice that are cocultured with ES cell-derived neurons serving as postsynaptic targets. ES cell-derived neurons are made by *in vitro* differentiation in embryoid bodies (EB) and are purified after EB dissociation by immunopanning. Axon outgrowth from neocortical explants leads to strong innervation of single, isolated ES cell-derived neurons. Preliminary data from recordings of miniature postsynaptic currents clearly show the establishment of both, excitatory glutamatergic and inhibitory GABAergic synapses on ES cell-derived neurons.

Second, we are studying the synaptic integration of GFP expressing, mouse ES cell-derived neurons that are added after purification by immunopanning as dissociated cells to organotypic slice cultures of rat neocortex. Preliminary morphological analysis showed development of typical dendritic arborizations.

In summary, these culture systems enable us to study functional synaptic integration and long-term synaptic plasticity in ES cell-derived neurons at different levels of complexity of the neocortical network.

715 Long-lasting enhancement of corticostriatal neurotransmission by taurine: Role of acetylcholine and dopamine

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The effects of taurine on corticostriatal neurotransmission were studied on slice preparations from mouse brain with the recording of neostriatal field potentials evoked by stimulation of cortical input. We have shown previously, that bath application of 10 mM taurine exerts a biphasic action on corticostriatal field responses: a fast and reversible inhibition, sensitive to both GABAA and glycine receptor antagonists followed by long-lasting enhancement (LLE) of synaptic efficacy, depending on the taurine transporter and sensitive to the strychnine (Chepkova et al, EJN 16:1523, 2002). As strychnine at 10μM can block GABAA, glycine and nicotinic acetylcholine receptors and taurine can excite cholinergic interneurons through glycine receptors we tested now the possible role of acetylcholine. LLE was prevented by both, muscarinic and nicotinic receptor antagonists scopolamine and mecamylamine, respectively. Presynaptic nicotinic receptors located at nigrostriatal afferents stimulate dopamine release; therefore we tested also the involvement of dopamine in LLE. The D1 dopamine receptor antagonist SCH23390 prevented the long-lasting enhancement of synaptic transmission by taurine. Involvement of PKC in the presynaptic regulation of dopamine release is in keeping with our finding that LLE is PKC-dependent. The data suggest that neuronal taurine uptake accompanied by a long-lasting modification of corticostriatal neurotransmission involves both cholinergic and dopaminergic mechanisms. Our result may be linked to the interesting finding, that there is a congruent decline of the levels of dopamine and taurine with aging.

Diffusional mobility of parvalbumin in spiny dendrites of cerebellar Purkinje neurons quantified by two-photon FRAP 716

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Ca^{2+}-binding proteins (CaBPs) represent key factors for the modulation of cellular Ca^{2+} dynamics. Especially in thin extensions of nerve cells, Ca^{2+} binding and buffered diffusion of Ca^{2+} by CaBPs is assumed to effectively control the spatio-temporal extend of Ca^{2+} signals. However, no quantitative data about the mobility of specific CaBPs in the neuronal cytosol are available.

We quantified the mobility of the endogenous CaBP parvalbumin (PV) in spiny dendrites of cerebellar Purkinje neurons with two-photon fluorescence recovery after photobleaching (FRAP). We first established and validated a quantitative analysis of FRAP data with experiments performed on fluorescein-labeled 10 and 40kDa dextrans and subsequently quantified diffusion of dye-labeled Parvalbumin. PV diffused readily between spines and dendrites with a median time constant of 49 ms (37-61 ms IQR). Based on published data on spine geometry, this value corresponds to an apparent diffusion coefficient of 43 $\mu m^2 s^{-1}$ (34-56 $\mu m^2 s^{-1}$). The absence of large or immobile binding partners for PV was confirmed in PV null-mutant mice.

Our data validate the common but so far unproven assumption that PV is highly mobile in neurons and will facilitate simulations of neuronal Ca^{2+} buffering. Our data suggest that PV will substantially modulate the spatio-temporal extend of neuronal Ca^{2+} signaling via buffered diffusion of Ca^{2+} and may be responsible for certain forms of heterosynaptic plasticity. Our experimental approach represents a versatile tool for quantifying the mobility of proteins in neuronal dendrites.

Modulation of the GABAergic transmission by different subtypes of nicotinic receptors in the rat inferior colliculus 717

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Anatomical and physiological studies suggest that acetylcholine plays a role in the processing of auditory signals in the inferior colliculus (IC). Our previous studies indicated that the modulation of GABAergic transmission in the IC of rats is a target of nicotinic acetylcholine receptors (nAChRs), but their exact function is still to be determined. The physiological effects of nAChR activation were characterized by applying the patch-clamp technique to neurons of acutely isolated IC slices of rats (P5-P12). In the presence of kynurenic acid (KYNA; 1 mM) and strychnine (0,5 µm) the nAChR specific agonists nicotine (10-50 µm; n=12), epibatidine (200 nM; n=21) and the β-

subunit-preferring agonists DMPP (100 μm; n=8) and cytisine (100 μm; n=7) significantly increased the frequency of GABAergic sPSCs. In addition, we observed inward currents in IC neurons which were activated by these nicotinic agonists (n=18). In contrast, the α7-specific agonist choline (10 mM) did not evoke any inward currents (n=9). It has been reported that strychnine (Sharples and Wonnacott, 2001) and KYNA could inhibit α7-homomeric nAChRs . However, in IC neurons 0.5 μm strychnine did not affect the nAChR-agonists-induced sPSCs frequency-increase. In contrast kynurenic acid strongly inhibited α7-subunit-containing nAChRs (n=8). In the presence of strychnine and DHbetaE (100 μm), a β-subunit-selective antagonist, the DMPP-induced frequency-increase was totally inhibited (n=5). In the absence of kynurenic acid the epibatidine-induced frequency-increase was only partially blocked by DHbetaE (n=3). In the absence of kynurenic acid, choline (10 mM) also increased the frequency of GABAergic sPSCs, but this effect was totally blocked by α-bungarotoxine (100 nM), an α7-specific antagonist (n=9). These data indicated the expression of at least two nAChR subtypes, an α7 and another subtype containing a β-subunit. In the presence of kynurenic acid and TTX (0.5 μm) epibatidine failed to increase the GABAergic sPSCs frequency (n=5). However, in the presence of DHbetaE (100 μm) and TTX epibatidine increased the sPSCs frequency significantly (n=3), suggesting that terminally located α7-nAChRs are involved in the modulation of the GABAergic transmission. We conclude that at least two different nAChR subunits (α7, β2/β4) contribute to the the modulation of the GABAergic transmission by nAChRs in the IC. The β-subunits-containing nAChRs appear to be located at preterminal sites of presynaptic GABAergic neurons as well as postsynaptically, while α7-nAChRs are expressed on the presynaptic terminals.

718 Muscarinic modulation of the GABAergic transmission in the rat inferior colliculus

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Presynaptic modulation is an important mechanism of the regulation of synaptic transmission. Muscarinic acetylcholine receptors (mAChRs) have been found to be involved in controlling the release of several neurotransmitters including GABA. We investigated the effect of muscarine on the GABAergic transmission in the inferior colliculus (IC), a relay station in the auditory pathways of mammals. By recording the synaptic activity in acutely isolated slices of the developing rat IC (P4-P14), we found that muscarine strongly increased the frequency of spontaneous postsynaptic currents (sPSCs) mediated by $GABA_A$ receptors ($GABA_A$Rs) without affecting their mean peak amplitudes. The muscarine effect disappeared in the presence of tetrodotoxin (500nM; n=7) indicating that the mAChRs involved were located at preterminal sites of presynaptic GABAergic neurons. The contribution of specific mAChR subtypes was investigated using subtype-selective drugs. Mecamylamine (0.01mM; n=5), a potent blocker of nicotinic AChRs did not affect the frequency of GABAergic sPSCs. The M1-antagonists telenzepine (50nM; n=6) and pirenzepine (0.01mM;n=5), and the M4 prefering antagonist himbacine (0.01mM;n=4) partially inhibited the muscarinic-induced PSC frequency increase. The M2 antagonist methoctramine (0.01mM;n=5) showed only a very weak inhibition

of the muscarinic effect on GABAergic sPSCs. In the presence of 4-DAMP (50nM), an antagonist preferring the M3 subtype, muscarine failed to increase the sPSC frequency in 6 IC neurons completely and in 3 cells nearly completely, indicating a contribution of the M3 subtype. The activation of mAChRs can evoke NO synthesis, an intracellular elevation of cGMP and an activation of cyclic nucleotide gated channels (Schobesberger et al. 2000, J. Neurophysiol. 83:1912-23), we tested the effects of NO donors. The application of Na^+ nitroprusside (SNP), L-arginine produced heterogenous results. In 6 out of 18 IC neurons the application of L-arginine (0.1mM; 10min; n=4) or SNP (1mM; 10min; n=2) increased the frequency of GABAergic sPSCs. The membrane permeable cGMP analogon 8-br-cGMP (0.1mM or 1mM; 10min; n=14) did not affect the GABAergic sPSCs. ODQ (0.01mM; n=2), an antagonist of the guanylate-cyclase, did not antagonize the muscarine-activated sPSC frequency increase. mAChRs also act by blocking M-type K^+ currents. Therefore, we tested the effect of muscarine on the K^+ currents of IC neurons (n=20), but found no change. We suggest that the activation of M3 AChRs depolarize GABAergic IC neurons, thereby increasing GABA release. In addition, we found some evidence that NO might have an effect on the GABA release in the IC. The complete mechanisms of the muscarine effect on the GABAergic transmission in the IC remains to be elucidated.

Diadenosine polyphosphates are hydrolyzed by members of the ecto-nucleotide pyrophosphatase/phosphodiesterase-family **719**

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Extracellular diadenosine polyphosphates (Ap_nA) are signaling molecules with numerous physiological functions. In the nervous system Ap_nAs were found in synaptosomal preparations of rat brain, in synaptic vesicles of *Torpedo*, and in chromaffin granules[1]. The release of diadenosine polyphosphates by regulated exocytosis leads to the activation of various purinoceptors in the central and peripheral nervous system. The action of Ap_nAs can be terminated by their enzymatic breakdown. Members of the E-NPP family hydrolyze phosphodiester and pyrophosphate bonds of various nucleotides. We characterized the ability of NPP1, NPP2 and NPP3 to hydrolyze diadenosine polyphosphates (Ap_nA, n = 3-5). Following transfection with the cDNA of human NPP1 and rat NPP3, hydrolysis of Ap_3A, Ap_4A and Ap_5A was measured on the surface of CHO cells or using membrane fractions. Human NPP2 was derived from vaccinia lysate. NPP1-3 degraded Ap_nAs asymmetrically, resulting in the formation of AMP and Ap_{n-1}. pH optima for hydrolysis of Ap_3A were at 8.5-9 for NPP1 and NPP3, and 10 for NPP2. The k_m values were in the low µmolar range and hydrolysis was activated by Mg^{2+} or Ca^{2+}. Comparison of the hydrolysis rates showed that NPP1-3 exhibited little preference

for Ap_3A, Ap_4A and Ap_5A. NPP1-3 were also capable of hydrolyzing the diguanosine tetraphosphate (Gp_4G). Hydrolysis of diadenosine polyphosphates (Ap_4A) by NPP1-3 was inhibited by the P2 receptor antagonists Cibacron Blue and PPADS. Suramin inhibited only the catalytic activity of NPP1 and NPP2, leaving NPP3 unaffected. Our results show that NPP1, NPP2, and NPP3 are major candidates for hydrolyzing dinucleoside polyphosphates in the nervous system and in other tissues and for controlling the receptor-mediated functions of the dinucleotides.

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[1]M. Teresa Miras-Portugal, Javier Gualix, Jesús Pintor (1998): The neurotransmsitter role of diadenosine polyphosphates. FEBS Letters 430, 78-82

720 Three-dimensional reconstruction of synapses onto thick tufted layer 5 pyramidal neurons in the rat somatosensory cortex

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Synaptic connections between neighboring layer 5 pyramidal neurons in rat somatosensory cortex appear anatomically homogeneous with respect to their morphology, number and distribution of synaptic contacts. However, synaptic efficacy as indicated by the mean somatic EPSP amplitude is highly variable between individual connections (range: 0.15-5.5 mV). Specifically, there is no apparent correlation between the number of anatomically identified synaptic contacts in a given connection and the mean EPSP amplitude, suggesting that these differences in synaptic efficacy could be due to differences in the number of release sites per contact, in release probability (since the variability of EPSP amplitudes also varies widely between connections), and in quantal size.

To look for possible anatomical correlates of these determinants of synaptic efficacy, three-dimensional reconstructions of synapses terminating on morphologically and physiologically identified layer 5 pyramidal neurons were made from serial electron micrographs. The quantification of surface areas and volumes of the pre- and postsynaptic structures including the number, distribution and size of pre- and postsynaptic densities and synaptic vesicles allows the definition of morphological correlates of functional parameters of synaptic transmission such as the ?readily releasable pool of synaptic vesicles? and the variability of quantal size.

Neurons were labeled either by Golgi impregnation of perfusion-fixed rats, or were biocytin-filled during whole-cell patch-clamp recording in acute slices. Serial ultrathin sections were cut at 60nm thickness and were photographed at a primary magnification of x13100. Negatives were scanned at 1200 dpi, aligned, and the contours of the pre-

and postsynaptic structures of interest as well as the surrounding neuropil were traced manually using the interactive software CAR. From the contours, three-dimensional surface reconstructions were obtained by Delaunay triangulation. Finally, the surface areas and volumes of the pre- and postsynaptic elements, including pre- and postsynaptic densities, were calculated. The number and spatial distribution of synaptic vesicles was also quantified.

So far five dendritic segments with impinging synapses were reconstructed. GABA postembedding immunohistochemistry revealed that only a minority (10-20%) of the synapses was GABAergic, suggesting that the majority of synaptic input is glutamatergic. Two types of excitatory synapses onto layer 5 pyramidal neurons can be distinguished: synapses with single, but large pre- and postsynaptic density, and synaptic contacts with multiple active zones. The pre- and postsynaptic densities are directly opposed across the synaptic cleft. We found that two pools of synaptic vesicles exist: one in close proximity (0?20nm) of the active zone with 2-5 docked vesicles probably representing the ?readily releasable pool of synaptic vesicles? at this synapse, and a second ?reserve pool? at a distance of approximately 100-150 nm.

The collection of data on the geometry of synapses and the surrounding neuropil is a prerequisite for realistic Monte Carlo simulation of various parameters of synaptic transmission such as the range of transmitter action, the saturation of postsynaptic receptors, and the involvement of transporters in shaping the kinetics of postsynaptic currents.

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Monitoring clathrin-mediated endocytosis in hippocampal synapses

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Various modes of vesicle recycling have been proposed for central nervous synapses, the major and best characterized pathway is the one mediated by clathrin.

Our aim is to understand the kinetics of clathrin-mediated endocytosis and how it can be modulated through different stimulation conditions.

In order to measure clathrin-mediated endocytosis as directly as possible we have cloned the clathrin light chain out of a rat cDNA library and fused its N-terminal with EGFP (EGFP-LCA1). When we overexpressed this construct in HEK-cells we could detect highly mobile sub-resolution particles at and close to the membrane. Immunocytochemistry showed a clear colocalization with the endogenous clathrin heavy chain as well as the adaptor protein 2 (AP-2). Neither punctuate staining nor colocalization were observed when expressing EGFP alone. Thus, EGFP-LCA1 seems to insert into structurally and functionally active coated pits.

To study clathrin-mediated endocytosis in central nervous synapses we overexpressed EGFP-LCA1 in neuronal cultures from hippocampus using the Semliki-Forest virus

system. Colabeling with the styryl dye FM 5-95, a red-shifted variant of the synaptic-activity marker FM1-43, by different stimulation conditions shows that the synapses are still capable of exo-endocytic activity. During and after stimulation we observed a transient redistribution of EGFP-LCA1 in presynaptic and axonal compartments. Superfusion with high potassium or electric field stimulation leads to a transient increase of the total fluorescence signal of EGFP-LCA1 in synaptic boutons accompanied by a transient fluorescence decrease in adjacent axonal segments. The bouton fluorescence rises after the stimulus onset and reaches amplitudes of up to 25% above resting value. This increase is reversible, decaying with a time constant of a few 10 seconds depending on stimulus duration and strength.

We suspect that the transient increase directly reflects the fraction of membrane bound clathrin at any time point. In this case, the signal would be directly proportional to the number of clathrin-coated pits and the rise and fall of the signal would be a measure of coat assembly and disassembly. To substantiate this model we assessed the diffusional speed of our construct in neuronal processes by various methods, and found that EGFP-LCA1 diffusion is not the rate-limiting step.

722 Visualization of Single Synaptic Vesicle Dynamics in Hippocampal Boutons

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Methods for tracking single synaptic vesicles in small synaptic boutons have not been available so far. We used high-resolution two-photon microscopy to visualize the dynamics of single fluorescently labeled synaptic vesicles in boutons of cultured hippocampal neurons. Application of the activity marker FM1-43 for 30 s to the bath solution in the presence of TTX allowed us to stain in most cases only one vesicle per bouton. FM1-43 was excited at 900 nm through a 1.2 N.A. objective mounted on an inverted microscope. The non-descanned fluorescence signal was detected with an avalanche photodiode in photon-counting mode. With integration times of 3-10 µs per pixel typically more than 100 photons were detected per vesicle per frame above an average background of ~1 photon per pixel. We visualized single vesicles for 10's of sec's at frame rates over 1 Hz without significant bleaching. This allowed us to collect more than 50 frames in one series. Images were further processed to remove high frequency noise as well as background structures. The relative position of the small vesicles was determined using a gaussian fit to the intensity distribution. The precision of the fitting procedure was found to be about 1 to 2 vesicle diameters as determined by measurements on boutons that were fixed with paraformaldehyde prior to measurements. We found that vesicle mobility within vesicle clusters is low, but also observed fast transport (~1 µm/s) of single vesicles.

Large dense-core vesicle secretion in the presence and absence of SNAP-25

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Large dense-core vesicles from chromaffin cells fuse with the plasma membrane in a Ca^{2+}-triggered process, which depends on the integrity of the neural SNARE complex, composed of synaptobrevin 2, syntaxin 1, and SNAP-25. Thus, cleavage of the SNARE proteins by neurotoxins abolishes fast Ca^{2+}-triggered exocytosis. Recently, a SNAP-25 null (or SNAP-25 deficient) mouse line was generated by targeted gene disruption (Washbourne et al., 2002, Nature Neuroscience 5, 19-26) in which evoked release at the neuromuscular junction and depolarization-evoked vesicular cycling in central neurons were abolished, but action potential independent, spontaneous release ('mini's') persisted. We wanted to study secretion in the absence of SNAP-25, and to establish a rescue approach using viral overexpression, which could be used to study mutations in SNAP-25 in a simple, null genetic background.

We prepared embryonic cultures of chromaffin cells from SNAP-25 knockout and control littermates and assayed secretion using capacitance measurements and amperometry. When stimulated by flash photolysis of caged-Ca^{2+} fast burst-like secretion from SNAP-25 knockout cells was absent, but a small amount of slower secretion was detectable. Short-term (8 hours) viral overexpression of SNAP-25 using a Semliki Forest Virus construct in knockout cells restored normal secretion, indicating that the phenotype was a direct result of the loss of SNAP-25. To study the remaining secretion in the absence of SNAP-25, knockout cells were dialysed to reach different $[Ca^{2+}]_i$ and secretion was quantified by counting amperometric spikes. SNAP-25 knockout cells displayed similar rates of release as controls at low $[Ca^{2+}]_i$ (<1µ-M calcium; control: 0.28±0.13 Hz; knockout: 0.19±0.10 Hz). At higher $[Ca^{2+}]_i$, both mutant and control cells showed an increased rate of release, but this rate was higher in the presence of SNAP-25 (~10 µ-M calcium, control: 2.79±0.43 Hz; knockout: 0.96 ± 0.19 Hz). These data indicate the persistence of Ca^{2+}-dependent secretion in the absence of SNAP-25, possibly through the participation of a constitutive SNAP-25 homologue, though at lower release rates. Single amperometric spikes from SNAP-25 knockout cells had similar risetimes, halfwidths, total charge, and peak amplitudes as control cells, indicating that the vesicular content and overall seretion was unaffected, but displayed a shorter duration of the foot signal (P=0.0011, 27 ±,+/+ cells, and 28 -/- cells analysed). This may indicate a shorter lifetime of the fusion pore in the absence of SNAP-25. Currently we are evaluating the effect of the SNAP-25 deficiency on vesicle docking using EM microscopy, and the involvement of SNAP-25 homologues in the remaining secretion from these SNAP-25 mutant chromaffin cells. Supported by the SFB 523 (E.N.), NIH 4-8989 (M.C.W.) and the Graduiertenkolleg 521 (G.N.).

724 Catecholamine secretion from chromaffin cells expressing wild type Synaptotagmin I, Syt II or phosphorylation mutants of Syt I only

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Synaptotagmins comprise a large protein family widely believed to be Ca^{2+}-sensors for Ca^{2+}-triggered exocytosis. The two vesicle-associated members synaptotagmin I (Syt I) and II are exclusively expressed in neurones or neurosecretory cells and are responsible for fast synchronised neurotranmitter release. *In vitro* binding assays showed that these two closely related isoforms bind phospholipids with distinct Ca^{2+}-affinities (~10 μ-M for Syt I and ~20 μ-M for Syt II, respectively; Sugita et al., 2002). Chromaffin cells express only Syt I and electrophysiological studies from Syt I knockout mice revealed a specific lack of fast burst-like secretion, whereas the slow burst of release persisted (Voets et al., 2001). Our goal was to compare neurosecretion from cells expressing Syt I or II. We also asked whether secretion could be regulated by phosphorylation of Syt I. We made chromaffin cell cultures from single new-born Syt I null mice and used viral overexpression techniques to study the effect of synaptotagmin isoforms or phosphorylation mutants in a clean genetic background. We combined capacitance measurements using the patch clamp technique with electrochemical detection of liberated catecholamine (amperometry) for evaluating exocytosis. Exocytosis was triggered by the flash photolysis of a Ca^{2+} cage included in the pipette solution. Two Ca^{2+}-sensitive fluorescent dyes were also introduced to measure $[Ca^{2+}]_i$ accurately.

Overexpression of Syt I in knockout cells reconstituted the missing fast burst of flash-evoked secretion. The time constant of the fast and slow burst of release was not different from wild type cells, but a significant increase in the amplitude of the fast burst component together with a similar decrease in the amplitude of the slow burst was observed. This means that introduction of more Syt I can shift the equilibrum between the Syt I-dependent and -independent releasable pools but can not increase the release rate. Overexpression of Syt II could also reconstitute the fast burst indicating that these two isoforms can replace each other. However, detailed kinetic analysis revealed that there was a significant increase in the exocytotic delay compared to overexpression of Syt I (5.8 and 12 ms, respectively) and the rate constant of the fast burst was also significantly reduced by a factor of 2. These data may indicate a reduced Ca^{2+}-affinity of the Ca^{2+} sensor for fast release in the presence of Syt II, and are in good agreement with previous biochemical studies of the affinities of calcium-dependent phospholipid binding.

We also studied the effect of all described phosphorylation sites in Syt I. PKC and CaMKII phosphorylate Syt I at Thr112, whereas caskII cause phosphorylation at Thr125 and Thr128 (Hilfiker et al., 1999). We constructed mutants that could mimic the constitutively phosphorylated state (by replacing Thr with Asp) or the unphosphorylated state (replacement of Thr with Ala) and overexpressed them in knockout cells. We found that

neither mutants of Thr112 nor double mutants of Thr125/Thr128 affected any of the kinetic components of release. Thus phosphorylation of Syt I does not seem to modulate exocytosis from mouse chromaffin cells.

Hilfiker et al. (1999) J. Neurochem. 73, 921—932.
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Functional analysis of mice deficient of the presynaptic active zone proteins piccolo and bassoon 725

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Active zones are the principal sites of transmitter release at conventional synapses of the CNS. Recently, several novel proteins that might play a role in active zone formation have been identified. Of these, the two largest ones, named piccolo and bassoon, are exclusively localized at the active zones. To assess their role in the active zone functioning, we have studied autaptic hippocampal cultures from bassoon and piccolo single and double mutant mice with patch-clamp and imaging techniques. Bassoon deficient neurons show a dramatic decrease in both, the amplitude of the evoked EPSC (53.8% decrease) and the size of the readily releasable pool (RRP, 52.6%) compared to wild-type littermates. However, short-term plasticity as well as vesicular and synaptic release probability remained unchanged. To further investigate this phenotype, we compared the density of functionally active synapses – identified by FM1-43 uptake and successive release – to the density of synapses identified by immunohistochemical staining with synaptophysin antibodies. We found no change in the density of immunohistochemically defined synapses, however neurons lacking bassoon showed a significantly reduced fraction of active synapses.

While in the piccolo single mutant mice we did not find a similar phenotye, electrophysiological analysis of neurons deficient of both proteins revealed an even more pronounced reduction in evoked EPSC amplitudes and RRP size (by 60 and 57,5%, respectively). However, no change in release probability nor short term plasticity was detectable, similar to the bassoon single mutant. Considering these results, we conclude that bassoon and piccolo are essential for the function of a certain subset of synapses.

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726 Complex synaptic connections of cholinergic antennal lobe projection neurones in the lateral horn neuropile of *Drosophila melanogaster*

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The lateral horn (LH) neuropile in insect brains represents a distinct high-order compartment of antennal lobe projection neurones (PNs). PNs in the brain of the fruit fly *Drosophila melanogaster* connect the primary sensory antennal lobe neuropile with the mushroom body (MB) calyces and finally terminate in the LH neuropile. In *D. melanogaster*, this area and the PN synapses it contains is indispensable for the instantaneous expression of behaviours induced by olfactory stimuli. We investigated within the LH neuropile the synaptic circuits of cholinergic PNs, immunoreactive to a choline acetyltransferase (ChAT) antiserum, by comparison with those in the MB calyces, using immuno light and electron microscopy. The spatial texture of presynaptic profiles shown by anti-synapsin immunoreactivity and of ChAT PN profiles were initially analyzed by laser scan confocal microscopy.

The LH neuropile occupies a much larger volume than the MB calyx neuropile and constitutes one of the so-called diffuse neuropiles of the brain that have so far eluded systematic investigation by anatomical means. The ChAT-immunopositive PN fibres of the massive inner antenno-glomerular tract extend into all portions of the LH neuropile, distributing in a non-random fashion. The extension and spatial density of presynaptic elements are much higher in the LH neuropile than in the MB calyces. Divergent presynaptic boutons, unlabelled as well as ChAT immunoreactive, appear to be the preponderant mode of synaptic input throughout the LH. Immunoreactive boutons accumulated in the lateral margins of the LH neuropile, whereas the more proximal region exhibited less intense immunolabelling. Synapses of ChAT-labelled fibres occurred either as divergent boutons having diameters of 1-3 μm, connected to unlabelled postsynaptic profiles, or as a minority of tiny postsynaptic spines having diameters of 0.05-0.5 μm among unlabelled profiles. Together these spines encircle unidentified presynaptic boutons of interneurones which occupy large areas of the LH. Synaptic contacts between LH ChAT elements were not observed. Synaptic circuits in the LH neuropile differ profoundly from those of the MB calyx, where ChAT-immunopositive spines were not encountered.

The LH neuropile may serve as an output area for the ChAT PN terminals, their boutons providing input to non-cholinergic relay neurones. The postsynaptic ChAT-immunoreactive neurites are an unexpected finding. If they arise from the PNs themselves, they may serve as indirect connections between PNs via unidentified local interneurones; PNs could as well receive other sorts of input from sources external to the LH neuropile.

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$GABA_b$-receptor-mediated modulation of Ca^{2+}-independent transmitter release in brain stem of neonatal mouse 727

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In brain stem respiratory network of mouse, $GABA_B$-receptor play a crucial role in rhythmic modulation from first postnatal day. Activation of $GABA_B$-receptors induced a G-protein-dependent modulation of both Ca^{2+} and K^+ channels. The $GABA_B$-receptor-mediated inhibition of presynaptic Ca^{2+} channels has been shown to play an important role in modulation of transmitter release. So far little is known about $GABA_B$-receptor-mediated modulation of Ca^{2+}-independent transmitter release. In the present study, the effect of $GABA_B$-receptor activation on Ca^{2+}-independent miniature and sucrose-evoked transmitter release was investigated in brain stem slices of neonatal mice (P0-P11) using whole-cell patch-clamp technique. In neurones of pre-Bötzinger-complex (PBC), spontaneous miniture PSCs (minis) were recorded in presence of 500 nM TTX. The frequency of minis increased with increasing postnatal age. Addition of 0.3 mM Cd^{2+} reduced the frequency of minis by 35% in P0-P3 mice, whereas it reduced the frequency by 65% in P4-P11 mice. Activation of $GABA_B$-receptor by 0.03 mM baclofen reduced the frequency of Ca^{2+}-independent minis by 50% in all tested mice. To further investigate the effect on Ca^{2+}-independent transmitter release, the neurones were superfused with sucrose (300 mM) in presence of TTX (500 nM). In these experiments, application of baclofen (0.1 mM) reduced the frequency of sucrose-evoked transmitter release by 40% in P0-P3 mice, while it reduced the mini-frequency by 55% in P4-P11 mice. Our results suggest that activation of $GABA_B$-receptor mediated a modulation of Ca^{2+}-independent transmitter release in brain stem respiratory network of neonatal mouse.

Neurexins as key modulators of synaptic Ca^{2+}-channel function 728

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Neurexins constitute a family of neuronal cell-surface molecules, which are encoded by three gene (Nx 1, 2, and 3). Each gene contains two independent promoters that drive transcription of a long (α-neurexin) and a short (β-neurexins) isoform, respectively. Although a involvement in synaptic function has been proposed for neurexins, so far there is no any direct experimental evidence for their role at synapses. Using a combined neurogenetic and electrophysiological approach, we have recently shown that mutant

mice lacking all three α-neurexin genes (triple KO) die shortly after birth caused by dramatic impairment of respiratory function. Electrophysiological analysis of neurons in the brainstem respiratory network revealed a functional deficiency of pre- and postsynaptic high-voltage activated calcium channels and, consequently, a dramatic reduction of spontaneous and evoked synaptic activity in triple KO mice. In another line of experiments neurexin 1α and 1β differing only in their extacellular portion were expressed in transgenic mice as fusion proteins tagged with horseradish peroxidase (HRP). We found that neurexins are specifically targeted to pre- but not postsynaptic specializations. Here, we report the analysis of a rescue approach by crossing the epitope-tagged HRP-neurexins into the null-mutant background. We demonstrate that presynaptic expression of HRP-neurexin 1α rescues the impairment of postsynaptic Ca^{2+}-currents. Specifically, the Ca^{2+}-current density improves by more than 180% in neurons of 2α-deficient mice (9.76 pA/pF in null-mutant compared to 17.35 pA/pF in null-mutant plus transgenic HRP-neurexin 1α), by 240% in 2α/3α- deficient mice (7.49 compared to 18.12 pA/pF), 200% in 1α/2α-deficient neurons (5.94 compared to 13.98 pA/pF). Finally, in triple KO mice, presynaptic expression of HRP-neurexin 1α improved the postsynaptic Ca^{2+}-current density by 160% (5.01 compared to 8.49 pA/pF). Transgenic expression of HRP-neurexin 1α not only improves postsynaptic Ca^{2+}-currents, but also - at least partially - rescues the impaired Ca^{2+}-dependent synaptic transmission in α-neurexin null-mutant mice. Remarkably, presynaptic expression of transgenic HRP-neurexin 1β that has the same regional and subcellular localization does not significantly improve the Ca^{2+}-channel function in null-mutant mice indicating that the extracellular domain may be responsible for regulating Ca^{2+}-channel activity. As a control for possible dominant-negative effects, transgenic expression of HRP-neurexin 1α or 1β did not alter Ca^{2+}-channel function in front of a wild type background. In summary, our data support the hypothesis that α-neurexins (but not β-neurexins) couple synaptic cell adhesion and recognition to Ca^{2+}-channels and potentially other signalling molecules.

729 Impact of Ca^{2+}-channels on the development of cochlear inner hair cells

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During postnatal development mouse inner hair cells (IHCs) pass through a period of spontaneous activity. This activity relies on the expression of CaV1.3 L-type Ca^{2+} channels. We studied IHCs of mice lacking the a1D subunit of Ca^{2+} channels and describe their presynaptic properties. The residual Ca^{2+} current consists of a dihydropyridine sensitive L-type component and a current that is probably mediated by SNX-482 insensitive R-type Ca^{2+} channels. The tiny remaining secretory responses are mainly mediated by L-type Ca^{2+} channels. As expected we did not observe any electrical activity in early postnatal mutant IHCs even when injecting large depolarising currents. Membrane potentials of mutant IHCs, however, displayed spontaneous inhibitory postsynaptic potentials up to at least p35. The voltage dependence of the underlying currents and their biphasic shape at depolarizing potentials characterizes them as potassium currents fol-

lowing Ca^{2+} influx through nicotinic receptors. We failed to observe such spontaneous or evoked inhibitory postsynaptic currents in IHCs from hearing control mice (p25). In line with previous studies (Kros et al., 1998) these cells displayed fast outward currents of large amplitude. On the contrary, mutant IHCs showed only slow outward currents, as if they were lacking the expression of large conductance Ca^{2+}-activated K^+ (BK) channels. The data suggest a complex developmental failure due to the strong reduction of Ca^{2+} channels-Supported by grants of the DFG and the MPG to T.M.

Presynaptic distribution of CAPS1 and CAPS2 implies a role in synaptic vesicle exocytosis 730

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Neural and endocrine cells employ regulated secretory pathways to release neurotransmitters and neuropeptides. While classical neurotransmitters are sequestered in small clear synaptic vesicles, neuropeptides are stored in large dense-core vesicles. However, both types of vesicles release their content via Ca^{2+}-dependent fusion with the plasma membrane. Calcium-activated protein for secretion (CAPS1) appears to be required only for large dense-core vesicle exocytosis. This 145 kDa protein was originally purified as a cytosolic factor required for Ca^{2+}-triggered dense-core vesicle exocytosis in permeabilized PC12 adrenal cells. CAPS1 has been proposed to act at a late post-docking stage in a dense-core vesicle specific exocytotic step just prior to, or at, the Ca^{2+} -dependent fusion step. To further elucidate the role of CAPS1 and a recently identified second isoform, CAPS2, we performed a detailed study of CAPS1 and CAPS2 distribution in different tissues of rat and mouse. Immunocytochemistry with polyclonal antibodies specific for CAPS1 and CAPS2 revealed the expression of CAPS isoforms in endocrine cells as well as in synapses of neurons, suggesting a role in large-dense core and synaptic vesicle exocytosis.

Two new complexin isoforms: CPX III and CPX IV 731

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Complexin I and II (CPX I and CPX II) are conserved proteins that bind to the assembled SNARE complex and thereby modulate neuronal exocytosis. Here we show that two further members of the Complexin family, CPX III and CPX IV, exist. They are highly homologous to each other but show only a limited homology to CPX I and CPX II. Westernblot analysis revealed that CPX III is expressed in brain and retina, whereas CPX IV is retina-specific. Using single and double-labeling immunocytochemistry and confocal laser scanning microscopy, we analysed the cellular and synaptic expression of

CPX III and CPX IV in the mouse retina. CPX III labeling was found in both synaptic layers of the retina and in the inner nuclear layer (INL). In the outer plexiform layer (OPL), the glutamatergic terminals of rods and cones, and in the inner plexiform layer (IPL) the glutamatergic terminals of bipolar cells and the processes of glycinergic amacrine cells express CPX III at their synapses. In contrast to that, CPX IV is expressed at glutamatergic synapses only. In the OPL, CPX IV is present in the synaptic terminals of rods but not cones, and in the IPL it is found in the terminals of a subpopulation of ON-cone bipolar cells. Our results show that in contrast to CPX I and CPX II, which are specific for GABAergic synapses of amacrine and horizontal cells, the expression of the new Complexin isoforms is restricted to glycinergic amacrine cell synapses and glutamatergic ribbon synapses of photoreceptor and bipolar cells. These differential and synapse-specific expression of the Complexins is likely to reflect differences in the presynaptic molecular machinery, responsible for the different modes of neurotransmitter release found in retinal synapses.

Biochemical experiments revealed that - like CPX I and CPX II - the new CPX isoforms bind to synaptic SNARE complexes. Furthermore, in overexpression experiments using hippocampal autaptic microdot cultures we found that CPX III or CPX IV overexpression rescues the weak synaptic transmission in CPX I/II double knock out neurons. These data suggest that the new CPX isoformes have a role in the Ca^{2+}-triggering step of neurotransmitter release which is similar to that of CPX I and II.

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732 Regulation of Alpha2A- and Alpha2C-Adrenoceptors in the Brain: Alpha2A Upregulation Persists after Chronic Psychosocial Stress

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Stress-induced activation of the central nervous noradrenergic system has been suspected to induce depressive disorders. Since episodes of depression often occur some time after a stress experience we investigated whether stress-induced changes in the α2-adrenoceptor system persist throughout a post stress recovery period. Brains of male tree shrews were analyzed after 44 days of chronic psychosocial stress and after a subsequent 10-days recovery period. Expression of RNA for α2A and α2C-adrenoceptors was quantified by *in situ* hybridization, and receptor binding was determined by *in vitro* receptor autoradiography.

Activities of the sympathetic nervous system and of the hypothalamo-pituitary-adrenal axis were increased during chronic stress but normalized during recovery. Alpha2A-adrenoceptor RNA in the glutamatergic neurons of the lateral reticular nucleus was significantly elevated after stress and after recovery (by 29 % and 17%). In the dorsal motor nucleus of vagus, subtype A expression was enhanced after recovery (by 33 %). In the locus coeruleus, subtype A autoreceptor expression was not significantly changed. Subtype C expression in caudate nucleus and putamen was elevated by the stress (by 5

and 4 %, respectively) but normalized during recovery. Quantification of 3H-RX821002 binding revealed receptor upregulation during stress and/or recovery.

Our data thus show (1) that chronic psychosocial stress differentially regulates expression of α2-adrenoceptor subtypes A and C, (2) that subtype A heteroreceptor expression is persistently upregulated whereas (3) subtype C upregulation is only transient. These findings coincide with post mortem studies in depressed patients revealing upregulation of α2A-adrenoceptors. They indicate a deficit in noradrenaline probably gradually acquired during the prolonged stress period and post stress.

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The Functional Role of the Complexin SNARE Complex Interaction

733

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SNAREs represent a conserved set of membrane-associated proteins that mediate exocytotic and intracellular membrane fusion events. Neuronal SNAREs include the synaptic vesicle protein Synaptobrevin/VAMP 2 and the two plasma membrane proteins Syntaxin 1 and SNAP-25. These proteins are able to form a thermodynamically highly stable, heat-, SDS- and protease-resistant complex, the core complex, which can be reversibly disassembled by the ATPase NSF and SNAPs. The assembly of the core complex is thought to be the essential step in the initiation of membrane fusion. Recent searches for regulators of core complex assembly and function using biochemical methods and yeast-two-hybrid technology led to the discovery of a large number of SNARE-interacting proteins. Among others, these proteins include the Complexins. Complexin I and its close homologue Complexin II form a family of small (15-16 kDa), soluble, acidic proteins that were initially discovered as stoichiometric components of the core complex. In contrast to many other SNARE interactors, Complexins bind only to the assembled core complex and not to its individual SNARE components. Studies on deletion mutant mice demonstrated that Complexins I and II are acting at or following the Ca++ triggering step of transmitter release by regulating the exocytotic Ca++ sensor, its interaction with the core complex fusion machinery, or the efficiency of the fusion apparatus itself.

Randomly mutagenated and site-directed mutated CPX 1 was tested for its core complex binding in biochemical assays. Several binding deficient CPX 1 mutants were identified and tested for functional activity. In contrast to the overexpression of wildtype CPX 1, SNARE complex binding deficient CPX1 failed to rescue the impaired EPSC amplitude and short term plasticity in autaptic cultures of CPX 1/2 KO hippocampal neurons. Recent structural data of the SNARE-Complex Complexin interaction revealed an a-helix N-terminal to the SNARE interaction sites. The function of this domain is completely unknown. We are currently, probing its function by disturbing the accessory a-helix with the insertion of several prolin residues and by deleting the N-terminal third of Complexin. Mutants will then be evaluated in biochemical and functional essays. In

summary, our results indicate that the binding of CPXs to the core complex is essential for its function.

734 Developmental expression of the Ca^{2+} binding protein Calretinin in calyx of Held nerve terminals

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It is known that the expression patterns of EF-hand proteins in the auditory pathway change during development. However, the functional role of these proteins in synaptic transmission, especially that of calretinin (CR), is still poorly understood. Among the three EF-hand proteins: calbindin, parvalbumin and calretinin, CR is the predominant EF-protein that is expressed in the calyx of Held nerve terminal (Lohman and Friauf, 1996, J. comp. Neurol.; Caicedo et al., 1996, Anat. Embryol.). The fast Ca^{2+} binding kinetics of CR and its dissociation constant for Ca^{2+} (0.5 μm to 1.5 μm) makes CR a suitable Ca^{2+} binding protein for buffering fast Ca^{2+} signals that are involved in synaptic transmission and synaptic short-term plasticity. Therefore, expression patterns of CR are likely to influence the transmitter release probability.

We investigated the expression pattern of CR in the calyx of Held during maturation of Wistar rats, using immunocytochemistry. Brain stem slices containing the region of the MNTB were stained simultaneous for Rab3A, a presynaptic marker protein, and CR. Rab3A monoclonal antibodies reliably stained all nerve terminals at all ages tested. In images where Rab3A and CR were overlain, the fraction of double-stained calyx of Held terminals was determined as the percentage of Rab3A labelled nerve terminals. We found that the fraction of nerve terminals expressing CR increased during development. In animals of postnatal day 6 (P6), no CR positive terminals were observed. In P14 the fraction of CR stained calyces was about 10% of the total Rab3A labeled terminals. At P21 about 60% of the nerve terminals were double stained, and this fraction further increased to 73% at P31.

Our findings indicate that synaptic maturation regarding CR takes more than three post-natal weeks. We propose that an increase in CR expression increases the Ca^{2+} buffer capacity in these nerve terminals. Since synaptic transmission and plasticity critically depend on Ca^{2+} homeostasis, CR might influence synaptic transmission and short-term plasticity.

735 Membrane potential has no direct effect on quantal release at a mammalian central synapse

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It is generally accepted that presynaptic intracellular Ca^{2+} concentration, $[Ca^{2+}]_i$, at the active zone determines the kinetics and amount of transmitter released (Ca^{2+} hypothe-

sis). An alternative hypothesis states that membrane depolarization, in addition to opening voltage-gated Ca^{2+} channels, further directly modulates the release machinery and thereby accelerates Ca^{2+} -induced transmitter release (Ca^{2+} voltage hypothesis). The calyx of Held nerve terminal is an ideal system to discriminate between these hypotheses, since both pre- and postsynaptic cell compartments are directly accessible to patch-clamp recordings and manipulation of the $[Ca^{2+}]_i$.

We made brainstem slices from 8-10 days old rats, which contained calyx of Held to MNTB principal neuron synapses. We performed simul-taneous pre- and postsynaptic whole-cell voltage clamp recordings combined with presynaptic Ca^{2+} uncaging. Presynaptic patch pipettes contained 1 or 1.5 mM DM-nitrophen and 100 μm Ca^{2+} indicator fura-2FF. Transmitter release was analyzed by deconvolution of excitatory postsynaptic currents (EPSC) measured in the presence of cyclothiazide and 1 mM external Ca^{2+}. Ca^{2+} currents were blocked with 200 nM ω-Agatoxin IVA and 1 μm ω-Conotoxin GIVA.

Under these conditions, Ca^{2+} uncaging induced normal transmitter release. Control responses were compared to a test condition in which the presynaptic holding potential was stepped from –80 mV to +80 mV 3 ms after the UV-flash. In examples from the same cell pair, in which the control and the test condition liberated similar amounts of $[Ca^{2+}]_i$, no apparent change in the EPSC was found. In more detail, for both conditions (n = 12) the transmitter peak release rates, the time-to-peaks and the delay times of the release rate versus flash elevated $[Ca^{2+}]_i$ (range: 1.5 μm - 8 μm $[Ca^{2+}]_i$) did not indicate a direct effect of membrane depolarization on transmitter release. Our findings suggest that at a mammalian fast central synapse, transmitter release is not influenced by membrane depolarization, and that the dynamics of the local $[Ca^{2+}]_i$, close to the active zone can explain the kinetics of transmitter release.

Presynaptic capacitance measurements and Ca^{2+} uncaging reveal sub-millisecond exocytosis kinetics and characterize the Ca^{2+} affinity of vesicle fusion at a fast CNS synapse 736

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Based on measurements of excitatory postsynaptic currents (EPSCs) and Ca^{2+} uncaging, the intracellular Ca^{2+} dependence of synaptic vesicle fusion has recently been reported for the calyx of Held CNS synapse (Bollmann et al. 2000, *Science*; R.S. & Neher 2000, *Nature*). To confirm these estimates in an approach independent of measurement of EPSCs, we combined measurements of presynaptic membrane capacitance (C_m) with flash-photolysis of a Ca^{2+} chelator (Ca^{2+} uncaging). This combination of techniques has allowed us to directly track the time-course of vesicle pool depletion with capacitance measurements at a CNS terminal.

Whole-cell patch-clamp recordings were made from calyces of Held in brainstem slices from P8–10 day rats, with pipette solutions containing the Ca^{2+} loaded chelator DM-nitrophen (DMN; 1.5 or 3 mM) and fura-2FF (0.1 mM), a low affinity Ca^{2+} indicator.

C_m was measured with the sine+dc technique (Pulse, HEKA-Elektronik), using a 70 mV peak-to-peak sine wave at 2 kHz. Light flashes with varying intensities elevated $[Ca^{2+}]_i$ to values of 2 – 55 μm. Presynaptic elevation of $[Ca^{2+}]_i$ lead to increases in C_m that were constant in an range of 10-55 μm $[Ca^{2+}]_i$ for a given cell, suggesting that a readily-releasable pool (RRP) of vesicles was depleted by $[Ca^{2+}]_i$-steps above 10 μm. However, these maximal responses varied between cells (coefficient of variation of 0.4), and had an average amplitude of 261 ± 127 FF (n = 55 responses). This cell-to-cell variability in the C_m response indicates that the size of the RRP varies substantially between terminals. Assuming a vesicular membrane capacitance of 65 aF (Sun & Wu 2002, *Nature*), this average change in C_m corresponds to a pool of about 4000 readily releasable vesicles. Depletion of the RRP with $[Ca^{2+}]_i$ steps above 10 μm was confirmed in cross-depletion experiments, in which flashes were combined with strong, pool depleting presynaptic depolarizations.

The kinetics of vesicle pool depletion were studied by fitting the rise in C_m with single- or double-exponential functions. In 44 out of 65 responses, single-exponentials described this rise in C_m well, with the remaining responses being well fitted by double-exponentials. The fast time constant of double-exponential C_m increases showed the same $[Ca^{2+}]_i$ dependence as the time constants of single-exponential rises, reaching an average of 2.7 ± 2.3 ms at 10-15 μm $[Ca^{2+}]_i$, and sub-millisecond values above 30 μm $[Ca^{2+}]_i$. Both the $[Ca^{2+}]_i$ dependence of the C_m amplitudes and of the release kinetics correspond well with a cooperative Ca^{2+}-binding model for the release machinery, which binds 5 Ca^{2+} before the vesicle fuses (R.S. & Neher 2000, Nature). For comparison, a time constant of pool depletion of 1 ms was observed in retinal bipolar cells and in cochlear inner hair cells at $[Ca^{2+}]_i$ of about 150 and 100 μm, respectively (Heidelberger et al. 1994, *Nature*; Beutner et al. 2001, *Neuron*). The fast kinetics of exocytosis at moderate $[Ca^{2+}]_i$ might represent an adaptation of action-potential driven synapses, which release neurotransmitter with high probability, causing a reliable activation of the postsynaptic cell.

737 The distribution and function of metabotropic $GABA_B$ receptors in spider peripheral mechanosensilla

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The spider exoskeleton has many mechanosensilla, innervated by bipolar neurons with their cell bodies close to the cuticle and their sensory dendrites attached to it. Numerous efferent fibers synapse with peripheral parts of the mechanosensory neurons, with glial cells surrounding the neurons, and with each other. Most of these fine efferent fibers are immunoreactive to an antibody against the inhibitory neurotransmitter γ-aminobutyric acid (GABA), and the sensory neurons respond to agonists of ionotropic GABA receptors with a rapid and complete inhibition (Fabian-Fine et al. J. Neurosci 19: 298-310, 1999; Panek et al. Eur J Neurosci 16: 96-104, 2002). In contrast, little is known about longer term GABA mediated effects. Here, we investigated the distribution and function of metabotropic $GABA_B$ receptors on spider leg mechanosensilla. The functional GA-

BA_B receptor comprises a heterodimer with two proteins, $GABA_BR1$ and R2. We used antibodies against both of these receptor proteins and found that anti-$GABA_BR1$ immunoreactivity was present in all parts of the sensory neurons, the efferent axons and the glial cells, but anti-$GABA_BR2$ labeling was restricted to the sensory neurons, and particularly to strongly labeled clusters on the distal parts of the sensory cell bodies. Western blot analysis confirmed that both $GABA_B$ receptor subtypes were present in spider central and peripheral nervous systems. Intracellular recordings from sensory neurons innervating the lyriform slit sensilla of the spider legs revealed that application of $GABA_B$ receptor agonists attenuated voltage-activated Ca^{2+} current and enhanced voltage-activated outward K^+ current, providing two separate mechanisms for controlling the neurons' excitability. These findings suggest that $GABA_B$ receptors are abundantly present in the spider central and peripheral nervous systems and that mechanosensory input in the peripheral sensilla can be finely tuned by $GABA_B$ receptor activation on the most distal parts of the sensory afferents. This would probably change the neuron's ability to detect different stimulus frequencies and amplitudes, and could cause a slow, sustained inhibition when the neurons are subjected to a repeated stimulus or when a change is required by behavioral circumstances.

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The action of Endophilin and the role of vesicle release by kiss-and-run at photoreceptor synaptic terminals in *Drosophila melanogaster* 738

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Endocytotic retrieval of presynaptic membrane occurs either by clathrin-mediated coated vesicle formation, or by so-called kiss-and-run. These two pathways can be differentially activated, depending on the rate of transmission. Synaptic terminals of *Drosophila melanogaster* photoreceptors R1-R6, with tonic, high-output release of neurotransmitter, offer important comparisons with the features of synchronized neurotransmitter release at the neuromuscular junction.

Both pathways of membrane recovery require Dynamin GTP-ase activity, and are arrested when temperature-sensitive mutant alleles of *shibire*, which encodes a Dynamin-like protein, are raised to their restrictive temperature (Koenig, Ikeda, 1996: JCB 135:797). Unlike kiss-and-run, the clathrin pathway additionally requires the action of Endophilin. Thus, the two pathways' relative contributions to endocytotic recovery can be distinguished from the mutants shi^{ts1} and $endo^1$, the actions of which we have investi-

gated on the R1-R6 terminals in the lamina neuropile beneath the compound eye. We also examined recovery from endocytotic blockade in the double mutant *shi*^{ts1}; *endo*^{1}. We used the *eyFLP* system (Newsome et al, 2000: Development 127:851) to create whole-eye mosaics in which all photoreceptors are homozygous mutant, in heterozygous flies. Such eyes establish a normal pattern of innervation of the lamina and underlying medulla.

Unlike *shi*^{ts1} and *shi*^{ts1}; *endo*^{1}, synaptic vesicles in R1-R6 mutant for *endophilin* were not significantly depleted, and remaining synaptic vesicles appeared darker. They possibly retained a partial clathrin coat in *endo*^{1} terminals and were thereby unable to re-enter the vesicle cycle. They clustered evenly in a cloud near capitate projections, specialized glial invaginations into R1-R6 terminals. Postembedding immunogold labelling revealed Endophilin expression at capitate projections, too, especially along the stalk and adjoining plasmalemma, not at the surmounting heads or elsewhere on the plasmalemma. The same regions also labelled with anti-clathrin immunogold, indicating that Endophilin marks sites of clathrin-mediated endocytosis in *Drosophila* R1-R6. Moreover, blockade of endocytosis also occurred at this site in *endo*^{1} flies, with multiple profiles of vesicles arrested in endocytosis along the capitate projection stalks.

Transients of the ERG, which arise from neurotransmission, were largely lost in flies with *endo*^{1} eyes, but the flies still exhibited phototaxis choice behaviour. Thus even when transmitter output is diminished in *endo*^{1} R1-R6 that lack a clathrin pathway, sufficiently to diminish the ERG transients, synaptic vesicles are still replenished from the kiss-and-run pathway at a rate sufficient to support exocytotic release, and together with possible long-term adaptation at central visual synapses, to support normal vision.

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739 Impact of Spontaneous Activity on Dendritic Properties of Neocortical Pyramidal Neurons In Vivo

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In neocortical and hippocampal pyramidal neurones, synaptic inputs are integrated both at the soma and in the dendritic tree, which contains active conductances supporting action potential backpropagation and enabling dendritic spike initiation. These properties have been studied principally in slice preparations, where they are strongly affected by focal synaptic stimulation. However there is typically less spontaneous synaptic activity in slices than *in vivo.* It is unclear how dendritic properties might change *in vivo* during periods of pronounced spontaneous activity and where in the dendritic tree spontaneous synaptic input occurs.

Using *in vivo* whole-cell recordings and 2-photon calcium imaging we have studied dendritic excitation during spontaneous synaptic activity in layer 2/3 neocortical pyramidal neurones in urethane-anaesthetized rats. Spontaneous activity was apparent as

short (several hundred milliseconds) periods of depolarized membrane potential (termed up-states) interspersed with quiescent periods (down-states). Spontaneous activity was completely blocked by local application of glutamate receptor blockers. Surprisingly, we observed no difference between input resistances in up- and down-states measured at the soma under current clamp. Membrane potential fluctuations were present in recordings from both the soma and distal apical tuft branches. In both cases they were synchronized with the electrocorticogram, indicating that proximal dendrites and distal apical dendrites receive spontaneous inputs more or less synchronously. The amplitudes of dendritic calcium transients induced by backpropagating action potentials were slightly larger during up-states, indicating that backpropagation is not blocked by spontaneous synaptic activity *in vivo*.

We conclude that spontaneous synaptic input occurs synchronously over large parts of the dendritic tree under urethane anaesthesia, altering the properties of both basal and apical dendrites. This may concurrently affect synaptic integration in different regions of layer 2/3 neurones shaping the integration of sensory input according to the state of the network.

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Purinergic modulation of synaptic activity and glia-neuron interaction in the cerebellum 740

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Glial cells communicate with each other by the release of substances like ATP or glutamate. Due to the close contact of Bergmann glial cells with neighbouring Purkinje neurons in the cerebellum, we used acute rat cerebellar slices (P14-P20) to study whether release of transmitters by glial cells may additionally modulate neuronal activity. We investigated the effect of activation of purinergic receptors on spontaneous postsynaptic currents (sPSC) in Purkinje neurons with whole-cell recordings. Application of ATP (0.1 mM, 3 min) increased the neuronal sPSC frequency by 91 ± 18% (n=19). The P2 receptor antagonist PPADS (10 μm) reduced the sPSC frequency by 23 ± 8 % (n = 11) and suppressed the ATP-induced increase. Adenosine (0.1 mM), ADP (0.1 mM) or the P2X agonist AMP-PCP (6 μm) had only minor or no effects, and did not mimic the response to ATP, suggesting that P2Y receptors mediated the ATP-induced increase. In double patch-clamp recordings from a Purkinje neuron and an adjacent Bergmann glial cell, stimulation of the glial cell (50 steps to +50 mV for 0.5 s, 1Hz) reduced the neuronal sPSC frequency by 36 ± 7% (n = 17; Brockhaus & Deitmer, 2002, J Physiol 545:581-93). This glial modulation of neuronal activity was suppressed during application of PPADS. Thus, we conclude that ATP acting on P2Y receptors is a modulator of neuronal activity in the cerebellum and plays a role in glia-to-neuron-signaling, perhaps via glial release.

741 Alpha-Neurexins determine transmitter release level at the mouse neuromuscular junction

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Neurexins represent a family of highly polymorph, neuronal cell-surface molecules that has been implicated in synaptic function. Using a neurogenetic approach we recently have shown that these proteins are localized at the presynaptic terminal, and that they are essential for efficient synaptic transmission. Mice lacking all three α-neurexin genes (triple knockouts) die shortly after birth from respiratory problems. Extensive cellular analysis demonstrated an apparent function for neurexins as organizer molecules of the activity of pre- and postsynaptic high-voltage activated calcium channels. In order to isolate the mechanisms governing the presynaptic influence of neurexins on synaptic transmission, we have now studied the neuromuscular junction (NMJ) as a model synapse. We have investigated the role of neurexins in neurotransmitter release at NMJs (which is mainly dependent on P/Q type calcium channels) of adult mice lacking two of the three α-neurexin genes. These mice have a severely reduced viability, they are hypomorph (double-knockouts weigh about half of littermate controls), and exhibit signs of motor system problems. Although the NMJs are only slightly reduced in size as shown by morphological means, their secretory function is severely impaired. The number of acetylcholine quanta secreted upon nerve stimulation (quantal content) and the frequency of spontaneous release events (miniature endplate potentials) were significantly decreased by about 40% compared to control NMJs, as demonstrated by intracellular micro-electrode recordings in nerve-muscle preparations. Interestingly, this synaptic phenotype manifests itself preferentially at NMJs in the soleus muscle (with slow-twitch fibres) rather than in diaphragm, which has a mixed population of slow and fast-twitch fibres.

742 Functional regions of the presynaptic cytomatrix protein Bassoon: Significance for presynaptic targeting and cytomatrix anchoring

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Exocytosis of neurotransmitter from synaptic vesicles is restricted to specialized sites of the presynaptic plasma membrane called active zones. A complex cytomatrix of proteins exclusively assembled at active zones, the CAZ, is thought to form a molecular scaffold that organizes neurotransmitter release sites. Here, we have analyzed synaptic targeting and cytomatrix association of Bassoon, a major scaffolding protein of the CAZ. By combining immunocytochemistry and transfection of cultured hippocampal neurons, we show that the central portion of Bassoon is crucially involved in synaptic targeting and CAZ association. An N-terminal region harbors a distinct capacity for N-myristoylation-

dependent targeting to synaptic vesicle clusters, but is not incorporated into the CAZ. Our data provide the first experimental evidence for the existence of distinct functional regions in Bassoon and suggest that a centrally located CAZ targeting function may be complemented by an N-terminal capacity for targeting to membrane-bounded synaptic organelles.

Postsynaptic recruitment of *Drosophila* LIN-7 to larval neuromuscular junctions depends on specific isoforms of DLG. 743

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The *Drosophila* tumor suppressor gene dlg-1 encodes a PSD-95/ SAP90-like membrane-asscociated guanylate kinase (MAGUK). Members of this protein family serve as central scaffolding molecules at various membrane specializations, including synaptic junctions. Previous studies have revealed that Dlg is required for proper localization of Shaker-type potassium channels, the cell adhesion molecule FasII and the PDZ-domain protein Scribble at larval neuromuscular junctions (NMJ). Both the multidomain structure of Dlg and the complex synaptic phenotype of severe dlg-mutant alleles, however, suggest that synaptic interaction partners of Dlg are by far more numerous. This notion is further supported by our finding that various Dlg isoforms bearing an aminoterminal L27-type domain (Dlg-S97) are predominantly expressed in synaptic regions within the CNS and at NMJs while they are absent from epithelial junctions. The corresponding aminoterminal region of SAP97, the closest mammalian homologue of Dlg, has been implicated in homotypic interactions with other scaffolding proteins, e.g. CASK/ mLin-2, which in turn bind Veli / mLin-7, a small PDZ domain protein. In this study we have evaluated the presence of DLin-7, the highly conserved homologue of mLin-7, in Dlg-based protein complexes. We found that both proteins colocalize at NMJs but segregate into different complexes in epithelia. Using genetic and transgenic approaches we could show, that the postsynaptic localization of DLin-7 at NMJs specifically depends on the presence of Dlg-S97 isoforms. Currently we are performing *in vitro* binding assays to figure out, whether the interaction between Dlg and DLin-7 is direct or mediated by other MAGUKs. To this end, our results exemplify the differential organisation of membrane-anchored protein complexes as a result of isoform-diversity.

744 Interaction of the Neuronal Calcium-Binding Protein Caldendrin with Postsynaptic Scaffolding Molecules

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We have identified a novel EF-hand calcium-binding protein named caldendrin, which is mainly associated with the somato-dendritic compartment of mature neurons in brain. Caldendrin is expressed in forebrain regions with a laminar cytoarchitecture. Within neurons, caldendrin localizes both to the cytosolic and to the cytoskeletal compartments with a clear enrichment in the post-synaptic density (PSD). It has a unique bipartite structure composed of a calmodulin (CaM)-homologous C-terminus and an unrelated N-terminal part. The C-terminal caldendrin structure determined by modeling perfectly fits the CaM structure suggesting common features and binding partners. However, some striking differences have to be considered: caldendrin harbors a longer hinge region between EF-hands 2 and 3 than CaM, possibly enhancing the flexibility of the two halves and has a different molecular surface. Moreover, evolutionary modifications of the caldendrin EF hand 2 presumably disturb Ca^{2+}-binding at this site, which in turn evolved as preferential protein interaction interface. The two-domain structure and the differential association with subcellular compartments suggest that caldendrin serves functions different from those of known neuronal Ca^{2+}-binding proteins like CaM. Caldendrin is most likely involved in dendritic and/or synaptic calcium signaling in a very precise spatio-temporal manner. Still the question remains how caldendrin is targeted and bound to the PSD. To identify potential binding proteins we perform Yeast-Two-Hybrid experiments and colocalisation studies in cultured cells with candidate synaptic scaffold molecules like SAP90/PSD95, SAP97 and SAP102. These membrane-associated guanylate kinases (MAGUKs) are considered as molecular organisers of the complex synaptic protein machines that can cluster neurotransmitter receptors or cell adhesion molecules and link them to the subsynaptic cytoskeleton. The hook-region of SAP97 is a known binding interface for CaM and molecular modeling suggests that caldendrin might bind to this region as well. First experimental evidence for an interaction between caldendrin and SAP90/PSD95 comes from co-immunoprecipitation studies from COS7 cells cotransfected with both molecules. Furthermore, caldendrin was shown to inhibit the G-protein coupled receptor kinase GRK5, which was recently demonstrated to play a role in the SAP90/PSD95-mediated anchoring of receptors at synapses.

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Rat GABA transporter 1 and 3: Functional analysis of EGFP fusion proteins and characterisation of a putative PET-ligand 745

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Inhibitory synaptic transmission in the CNS is terminated by removal of γ-aminobutyric acid (GABA) from the synaptic cleft by a cellular uptake system consisting of high-affinity GABA transporter proteins (GAT). Uptake of each GABA molecule is accompanied by cotransport of two Na^+ and one Cl^-, hence the action of GATs is electrogenic. The predominant GATs in the mammalian CNS are designated GAT-1 and GAT-3, which are believed to represent the neuronal and glial transporter, respectively. Both are pharmacologically distinct drug targets for the therapeutic intervention of epilepsy as well as targets for selective Positron Emission Tomography (PET) ligands to non-invasively visualise the activity of the inhibitory system *in vivo*.

For cloning of GAT-1 and GAT-3 RNA was isolated from rat brain tissue. The complete coding region of both rGATs was reverse transcribed into cDNA and amplified by PCR. The reaction products were ligated to a cloning vector, analysed by DNA sequencing and further subcloned into an eukaryotic expression vector. We constructed a N-terminal fusion of each transporter to the *enhanced green fluorescent protein* (EGFP) by subcloning a slightly truncated cDNA into the pEGFP-N1 vector.

HEK 293 cells were transfected with the plasmids and the distribution patterns of the rGAT-EGFP fusion proteins were analysed 24 h and 48 h after transfection by epifluorescence microscopy. Accumulation of fluorescence indicated clustering of rGAT-1-EGFP in the plasma membrane. In contrast, rGAT-3-EGFP proteins were uniformly distributed in the plasma membrane.

Functionality of the recombinant GATs was tested with the whole cell configuration of the patch-clamp technique. In HEK 293 cells transfected with rGAT-1 and rGAT-1-EGFP DNA as well as in rGAT-3 and rGAT-3-EGFP transfected cells GABA induced fast transient chloride currents. The normalised dose-response curves for both rGAT-1 types displayed no significant differences. Currents were detectable at 1 µm GABA and reached maximal amplitudes at 3 mM GABA, with similiar EC_{50} values of 82.1 ± 16.2 µm for rGAT-1 versus 76.2 ± 11.8 µm for the fusion protein. GABA dose respond curves of both rGAT-3 proteins showed significantly higher GABA sensitivities with starting points of 0.1 µm and maximal activity at 100 µm. Though these characteristics were similar for rGAT-3 and rGAT-3-EGFP, their EC_{50} values were significantly different with values of 1.89 ± 0.11 µm and 3.65 ± 0.34 µm GABA, respectively.

Analysis of the putative subtype-specific GAT inhibitor (±)-1-[2-(Triphenylmethoxy)ethyl]-3-piperidinecarboxylic acid (SNAP 5114) and its fluoro derivative F-SNAP 5114 was performed on rGAT-1-EGFP and rGAT-3-EGFP. For rGAT-1 SNAP 5114 inhibited only active transporters and reduced current amplitudes down to 7 ± 2.3% at 3 mM with an IC_{50} of 454 ± 16 µm. In case of rGAT-3, SNAP 5114 displayed a much higher sensitivity with an IC_{50} of 3.6 ± 0.48 µm accompanied by a current reduction down to 17 ± 3%. In contrast, the F-conjugated SNAP derivative failed

to inhibit rGAT-1 transporter currents and showed a reduced inhibition of 20 ± 4% with a slightly decreased sensitivity (IC_{50}: 8.7 ± 0.8 μm) for rGAT-3.

746 Activity-Dependent Morphological Plasticity in Hippocampal Neurons

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The ability of neurons to change strength and number of their synaptic connections represents a potent mechanism for long-term information storage. Dendritic spines are prominent sites for morphological plasticity, whose turnover is under developmental as well as activity-dependent control. Since spines carry a large fraction of the excitatory input to hippocampal pyramidal neurons, the growth of new spines marks candidate sites of de novo synaptogenesis. Our aim was to first establish a model system for activity-dependent spino/synaptogenesis in the central nervous system, which would then allow us to study the underlying molecular mechanisms as well as the functional consequences of newly generated spines/synapses.

At first we implemented an experimental set-up based on time-lapse two-photon microscopy to faithfully image and record neuronal morphology. Organotypic hippocampal slices from Thy-1-GFP-transgenic mice were used, since they offer a sparsely labeled neuronal network ideal for two-photon imaging. We identified and tracked over time presynaptic boutons and postsynaptic spines, which are morphological hallmarks of excitatory synapses between CA3 and CA1 neurons in the hippocampus. We then implemented an experimental paradigm to induce growth of new spines by synaptic activation. Morphological changes, indicative of new filopodia as well as spines, in CA1 neurons can be observed following local extracellular tetanic stimulation. Spine growth occurred on a timescale of minutes and typically started within 10 to 20 min after stimulation. In most cases they persisted for the duration of the experiment (up to 4 hours). To investigate whether and when these spines become functional synapses, we re-identified these structures after fixation, so as to check them immunohistochemically and at the EM level for synaptic markers and specializations. In case the new spines indeed mature into functional synapses, we hope to be able to investigate synaptogenesis, i.e. its temporal orchestration and molecular assembly, at the level of single and identified synapses.

Hippocampal LTP requires pre- and postsynaptic TrkB signaling

747

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Long-term potentiation (LTP) in the Schaffer collateral/CA1 pathway has been shown to be dependent on the neurotrophin brain-derived neurotrophic factor (BDNF). In the present study we set out to test whether the action of BDNF and its receptor TrkB was presynaptic or postsynaptic or both. Despite a well-documented involvement of TrkB signaling in synaptic plasticity, its exact site of action has not been determined conclusively and studies indicating pre-, as well as postsynaptic mechanisms have been published. Clearly, BDNF can presynaptically increase transmitter release and acutely enhance glutamatergic synaptic transmission. However there are also potential postsynaptic target molecules for TrkB signaling such as the NMDA and AMPA type glutamate receptors, and calcium binding proteins.

Our experiments attempted to test the pre- or postsynaptic contribution of TrkB signaling by interfering with phospholipase C γ (PLCγ)-signaling. PLCγ binds to the activated TrkB-receptor, translocates to the plasma membrane and cleaves PtdIns(4,5)P2 into DAG and IP3. IP3 binds to IP3 sensitive receptors (IP3R) on internal Ca^{2+} stores, releasing Ca^{2+} into the cytosol. It is this TrkB pathway that seems to be mostly involved in activity dependent synaptic plasticity (Minichiello et al. 2002).

PLCγ translocation is critically dependent on a specialized module at the N-terminus of the PLCγ protein, called the pleckstrin homology (PH) domain. When overexpressed the PH domain alone functions as a dominant negative competitor for PLCγ activity and thereby interrupts PLCγ-mediated signaling (Falasca et al., 1998). To determine the pre- and/or postsynaptic action of TrkB signaling we analysed synaptic plasticity in the form of extracellular LTP in acute hippocampal slices that were infected with recombinant Sindbis viruses coding for the PH domain of PLCγ (Sin-PH-EGFP). Local microinjection of Sin-PH-EGFP viruses and transduction of pyramidal cells in the CA1 and/or CA3 area of the hippocampus confined interruption of PLCγ mediated signaling to either the pre- or postsynaptic side of the Schaffer collateral/CA1 connection.

Our experiments show that the PH domain had to be expressed pre- and postsynaptically to achieve a significant reduction in LTP magnitude. Interruption of PLCγ activation in the CA1 or CA3 region alone had no effect on the induction or expression of LTP. This entails the surprising consequence that the BDNF/TrkB pathway acts on both, the pre- and postsynaptic side of a neuronal connection in order to achieve strengthening of the invervening synapse(s). It seems as if pre- and postsynaptic pathways contribute to LTP in an at least partly redundant fashion and only loss of both pathways together abolishes BDNF mediated synaptic plasticity.

748 Evidence for ephaptic feedback in mossy fiber-CA3 synapses: Positive correlation between paired responses

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We tested the Byzov's hypothesis of on positive intrasynaptic ephaptic (electrical) feedback (EFB) in central synapses. The EFB should appear in synapses with large enough synaptic gap resistance (Rg) due to local depolarization of presynaptic release sites by the excitatory postsynaptic current (EPSC) flowing via Rg and resulting increase in transmitter release probability. A novel computer model of a synapse with EFB was developed *(www.iitp.ru/projects/eq/).*The model incorporates quantal transmitter release from multiple release sites and presynaptic paired-pulse facilitation. Simulation experiments revealed a new feature of paired-pulse interaction, namely a positive correlation between the amplitudes of the first and second paired EPSCs when EPSC2 partially overlaid EPSC1 and Rg was large enough. A clear demonstration for such correlation was an increase in the mean amplitudes of EPSC2 evoked after "successes" of EPSC1 as compared to EPSC2 evoked after EPSC1 failures. The magnitude of the increase positively correlated with the degree of the EPSC2/EPSC1 overlay. To test the predictions of the above computer experiments we recorded "minimal" EPSCs from CA3 pyramidal neurons of rat hippocampal slices. The EPSCs were evoked by stimulation of the mossy fibers that form giant synapses with presumably large Rg. All above predictions were confirmed. There was a positive correlation between the amplitudes of EPSC2 and EPSC1. The correlation was expressed in increased average amplitudes of EPSC2 superimposed on EPSC1 successes in comparison with average amplitudes of EPSC2 evoked after the smallest first responses (mostly EPSC1 failures). There was a strong positive correlation between the magnitude of such increases and extent of EPSC2/EPSC1 overlay. In the same synapses, a "supralinear" effect of postsynaptic hyperpolarization was found: EPSC1 amplitude increased for >2 times with 50% holding potential shift (from -60 to –90 mV). This effect has been previously described in physiological experiments and represents a characteristic consequence of EFB as it was further confirmed here with the new model. In general, the data are compatible with the presence of the positive EFB in large central excitatory synapses and incompatible with an alternative "chemical" hypothesis. The latter explains the "supralinear" effect of postsynaptic hyperpolarization by a suppression of a potential-dependent release of a hypothetical inhibitory retrograde messenger from the postsynaptic neuron. However, this hypothesis can not account for the positive correlations found above. EFB should essentially increase the efficacy of large central synapses, especially when they are activated at short intervals. A high frequency activity is typical for the hippocampus during animal behavior related to cognitive functions. Supported by Wellcome Trust, INTAS and RFBR.

Competitive Interactions between Potentiated Synapses 749

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Hippocampal Long-Term Potentiation (LTP), a prominent model for learning and memory, consists of at least two phases, an early (E-LTP) and a late phase (L-LTP), the latter of which is dependent on de novo protein synthesis. L-LTP can be disrupted by blocking protein synthesis at the time of induction while later drug application (3 to 4 hours after induction) has no effect. It has been suggested by Frey and Morris (1997) that proteins synthesized upon L-LTP induction can be "captured" such that E-LTP is converted into L-LTP using these proteins. This model predicts that two pathways might interact in a competitive fashion if the pool of available proteins were limited, for example by protein synthesis blockade. Another prediction is that LTP in a second pathway could act as a sink for an otherwise abundant protein uncovering an effect similar to that of protein synthesis inhibition on L-LTP.

In order to test this "synaptic competition" hypothesis, we used acute hippocampal slices from wistar rats. LTP was induced associatively by simultaneous weak and strong tetanization of two independent sets of Schaffer collaterals projecting from the CA3 to the CA1 area. The associative nature of LTP ensured that L-LTP was induced in both pathways. Four hours after induction one of the pathways received a second tetanus (reactivation) in the presence of a protein synthesis inhibitor, anisomycin. As predicted by the synaptic competition hypothesis, tetanization of the "weak" pathway induces a decay of the potentiation in the "strong" pathway, and the degrees of potentiation and decay are quantitatively correlated. This indicates that "plasticity proteins" synthesized by the initial strong tetanization can be captured by the weakly potentiated synapses at the expense of the strong pathway. This interpretation is corroborated by the observation that reducing the time between induction and reactivation abolished the effect, whereas increasing the duration of drug application increased the observed effect. In the absence of anisomycin no decrease was observed. These data are best explained by a redistribution of a limited pool of proteins synthesized upon LTP induction among potentiated synapses. This provides further support for the synaptic tagging hypothesis and constitutes a mechanism of "synaptic competition" by which the cell might be able to regulate overall synaptic strength.

A direct role for truncated TKRB receptors in glial calcium signaling 750

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Neurotrophins such as brain-derived neurotrophic factor (BDNF) are trophic factors that regulate the survival and differentiation of neurons. BDNF is also crucially involved in the modulation of synaptic plasticity and causes fast excitation of central neurons and

glial cells (Kafitz et al., Nature 401, 1999; Rose et al., Pflügers Arch, 2002). BDNF exerts its effects mainly through tyrosine kinase receptors ($TrkB^{FL}$), which can activate several intracellular signalling cascades. Many cells also express splice variants of $TrkB^{FL}$ that lack the intracellular tyrosine kinase domain (truncated TrkB). So far, no direct signalling role for these truncated receptors has been demonstrated. The mechanisms of BDNF-evoked neuronal excitation have recently been elucidated: it is caused by activation of $TrkB^{FL}$ and subsequent opening of Na^+ channels (Blum et al., Nature 419, 2002). The present study aimed to identify the cellular mechanisms of BDNF-evoked activation of glial cells.

Brief application of BDNF (25 ng/ml, 20-100 ms) by a focal ejection pipette induced Ca^{2+} transients in cultured rat hippocampal astrocytes. The BDNF-evoked Ca^{2+} signals persisted in Ca^{2+}-free saline and were blocked by the SERCA-inhibitors CPA or thapsigargin, as well as by the IP_3 receptor antagonist 2-APB and by U73122, a blocker of phospholipase C. This strongly suggests that they are mediated by Ca^{2+} release from internal stores following activation of phospholipase C and production of IP3. The neurotrophin response profile indicated that the BDNF-induced glial Ca^{2+} signals are mediated by TrkB-receptors. The Ca^{2+} signals were unaffected by the tyrosine kinase blocker K-252a, and persisted in TrkBFL-knockout mice. Quantitative RT-PCR and immunocytochemical stainings revealed that the glial cells express high levels of truncated TrkB-T1 receptors, whereas $TrkB^{FL}$ and TrkB-T2 are expressed in minute amounts. Transfection with TrkB-antisense vectors caused a reduction of the BDNF-induced glial Ca^{2+} signal by about 50%.

Thus, our results demonstrate that the fast activation of glial cells by BDNF is mediated by different mechanisms than that of neurons. Furthermore, they indicate that BDNF-evoked glial Ca^{2+} signals are caused by activation of truncated TrkB receptors and suggest a novel role for these receptors in intracellular Ca^{2+} signalling.

751 Fluorescently tagged EphB2 receptors to study their dynamics in neurons.

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Eph receptors and their ligands are key players in many cellular processes during neuronal development. Recent evidence strongly suggests that Eph receptor tyrosine kinases and their ligands also contribute to synapse formation and synaptic plasticity. EphB2 receptors are found to be highly enriched in synapses and to specifically interact with the NR1 subunit of the NMDA receptor (NMDAR). Furthermore, clustering of the receptors and interaction with NMDAR can be rapidly induced upon stimulation with ephrinB ligands, possibly mimicking initial steps of excitatory synapse formation. Also, EphB2 receptor activation leads to phosphorylation of syndecan-2, a protein that is involved in spine formation and spine morphogenesis. Moreover, our lab and others could demonstrate that lack of EphB2 leads to impairment of two forms of synaptic plasticity in the hippocampus such as long-term depression and long-term potentiation. These initial findings strongly point towards the importance of this receptor family in

plasticity and synapse formation, however, the details of the mechanisms still remain unclear.

In order to better understand the role of EphB2 receptors during these processes, we fluorescently tagged EphB2 receptors to study their dynamics of trafficking, insertion and clustering in neurons. We generated chimeric proteins of EphB2 receptors fused to one of the variants of the enhanced green, blue or yellow fluorescent protein E(G/C/Y)FP. ExFP was inserted in one of three different positions of the EphB2 receptor, the N-terminus, a site adjacent to the juxtamembrane region or in between two functional relevant domains of the cytoplasmic part of EphB2. We successfully transfected and expressed chimeric EphB2 receptors in HEK293 cells and neurons, which showed intense fluorescence at the membrane. Biochemical methods were used to test functionality of the fusion protein concerning tyrosine phosphorylation and interaction with known partners. We observed that kinase activity and recruitment of certain intracellular interaction partners (e.g. NR1 & GRIP2) appeared not to be impaired. Furthermore, we looked at the dynamics of the EphB2-ExFP proteins in the membrane of dissociated hippocampal neurons upon exogenous stimulation with its ephrinB ligand. Immunohistochemistry and timelapse imaging of young cultured neurons (DIV3-transfection+3days-wait) demonstrated that ephrinB application induced a dramatic increase in cluster size and number within a few minutes. This increase was less pronounced in older cultures (DIV6+3). Without any exogenous stimulation older neurons showed a higher number of clusters compared to young neurons. This suggests that endogenous ligands provided by the interaction with other cells may also induce formation of EphB2 clusters, which might be associated with synaptic sites. In the future, we will look at the timing of EphB2 cluster formation in relation to synapse formation using the EphB2-ExFP fusion proteins.

Action potential and ryanodine evoked calcium rises in synaptic terminals of cerebellar basket cells

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Using two-photon confocal scanning microscopy in conjunction with whole-cell recordings in cerebellar slices of young rats, we imaged calcium-dependent fluorescence transients in the axonal terminals made by cerebellar basket cells onto Purkinje cell somata. We compared the presynaptic action potential (AP) evoked calcium transients with the Spontaneous Calcium Transients(SCaTs) obtained in the presence of ryanodine, after block of APs by tetrodotoxin. The calcium-sensitive fluorescent indicator fluo-4 was introduced in the basket cell through the patch-clamp pipette, at a concentration of 400 μm. Basket terminals were identified by co-localization of the two-photon basal fluorescence image of the presynaptic axon with the transmitted light image of the Purkinje cell soma. Trains of 4 APs (50 Hz) were evoked by short intracellular depolarizations of the basket cell soma. Calcium-transients were analyzed in terms of the percentage change in fluorescence with respect to the pre-stimulus period (% DF/Fo). In five terminals, the AP-evoked transients had an average peak amplitude of 315 ± 142 %

(mean ± s.d.). The decay was approximated by a single exponential with average time constant of 3.7 ± 1.9 sec. In the same terminals, ryanodine induced SCaTs. Single spontaneous events were characterized by a highly localized site of origin from which the signal diffused into the neighboring axonal regions. The relative change in fluorescence was measured for all pixels along the axonal tract and the source of the signal was identified as the site with the fastest rise time. The amplitude and decay time were measured at the source. The average SCaT amplitude was 88 ± 12 %. The average decay (through an exponential fit) was 2.2 ± 0.9 sec. Larger events with a complex rise phase were often observed and could be attributed to high frequency bursts of summating single events.

753 Glutamate - mediated cell - death in epidermal cells of *Xenopus laevis*

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Neurotransmitters and their receptors have also been localized outside the nervous system. E.g., in keratinocytes, acetylcholine, acetylcholine - receptors, and acetycholine - metabolizing enzymes have been identified (Grando, J Investig Dermatol Symp Proc 1997, 2: 41-8). However, acetylcholine receptors are not the only neurotransmitter - receptors expressed in the epidermis. Recently, in the human epidermis glutamate - receptors have been identified in the basal layer (Morhenn et al., Eur J Pharmacol 1994, 268: 409-14). We hypothesized that glutamate may be involved in apoptosis of epidermal cells. This hypothesis was tested using immunocytochemistry and apoptosis assays using *Xenopus laevis* as a model. The skin of amphibians has attracted much attention, because of its hormone - dependent changes during metamorphosis.

Immunostaining of whole - mounts and tissue sections of *Xenopus laevis* embyros revealed numerous epidermal glutamate – immunoreactive cells. Most immunoreactive – cells have a polygonal shape and, typically, are separated from each other by at least one unlabelled cell. Glutamate – immunoreactive material is either homogeneously distributed throughout the cytoplasm or it is concentrated at the apical area of the cell. In the skin of adult frogs, immunoreactive – cells are apparently randomly distributed within the epidermis or show a stereotype arrangement between neuromasts.

Next, we exposed Xenopus embryos to different concentrations of glutamate (100 µm, 300 µm, 1 mM) and quantified epidermal cell death. To quantify cell death of epidermal cells, embryos have been incubated either with Hoechst 33342 to visualize fragmented nuclei, which are a hallmark of apoptotic cells, or fragmented DNA has been labeled using a TUNEL (TdT – mediated dUTP digoxigenin nick end labeling) protocol. Exposure of Xenpus embryos to different concentrations of glutamate increased the number of fragmented nuclei and increased the number of TUNEL - stained cells.

In conclusion, our experiments reveal an epidermal cell specific localization of glutamate and, furthermore, that glutamate triggers apoptosis in amphibian epidermal cells.

Nitric oxide and cGMP - mediated modulation of Ca – and KCa - conductances in snail neurons 754

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The mechanism of nitric oxide (NO) - dependent modulation of Ca^{2+} - and Ca^{2+} - activated potassium (KCa) conductance was studied in identified subesophageal neurons of the pulmonate snail, Helix pomatia. KCa is voltage as well as Ca^{2+} - dependent and sensitive to charybdotoxin (ChTX), Ba2+, and tetraethylammonium (TEA), but insensitive to aminopyridine (4 – AP). Thus, the KCa – current shows similarities to large-conductance (BK) channels. NO - donors (sodium nitroprusside, SNP; S – nitro – N – acetylpenicillamine, SNAP) slowly decreased the current amplitude. Decline of the current amplitude by NO - donors was qualitatively mimicked by a membrane – permeable cGMP analogue (dibutyryl – cGMP, db- cGMP). Methylene blue, an inhibitor of guanylyl – cyclase, or erytho – 9 – (2 – hydroxyl – 3 – nonyl) adenine (EHNA), an inhibitor of the cGMP – stimulated phosphodiesterase 2, prevented NO - donor – induced decrease of KCa - current.

The L – type Ca^{2+} - current either did not respond to NO – donors or increased following application of NO – donors. Exposure of neurons to db – cGMP increased the current amplitude. In EHNA pretreated neurons, the L – type Ca^{2+} - current did not respond to NO or cGMP.

Our experiments demonstrate that the NO – mediated effects on Ca^{2+} - and KCa – conductance in snail neurons dependent on the generation of cGMP and subsequent cGMP – dependent enzymes.

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Identification and functional characterization of monomeric GTPases, which bind to the GDP/GTP exchange factor collybistin 755

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During neuronal development, the peripheral membrane protein gephyrin is essential for anchoring the inhibitory glycine receptor and GABAA receptors at subsynaptic areas. Gephyrin couples the structural β subunit of the glycine receptor to cytoskeletal elements including microtubules and microfilaments in a multi-step process, which includes additional regulatory proteins. The GDP/GTP exchange factor collybistin was shown to interact with gephyrin thereby possibly regulating the activity of small GTPases of the Rho family such as Rho, Rac and CDC42. This family of monomeric GTPa-

ses, which can be considered as “molecular switches” depending on their GTP content are known to regulate the cell shape by changing the polymerization state of intracellular actin. Therefore, collybistin and monomeric GTPases of this family might be implicated in establishing the appropriate cell morphology during synaptogenesis and/or dynamically mediate or react to changes in synaptic plasticity. The primary structure of collybistin displays a PH/DH tandem domain, which is characteristic for the family of dbl-like GEFs. Therefore collybistin could link synaptic transmission to cytoskeletal rearrangements. To identify proteins that interact with collybistin, we screened a human embryonic brain library with the yeast two-hybrid system using collybistin as bait. We identified 4 independent cDNA clones, which encode putative interacting proteins. One of these encoded a monomeric GTPase of the Rab family with yet unknown functions. Immunoprecipitations from transfected cells and from brain extracts demonstrated that collybistin can form complexes with both the Rab GTPase and with CDC42. Similarly, in cultured embryonic rat spinal neurons collybistin colocalized with the Rab GTPase and with CDC42. Moreover, we could demonstrate that recombinant collybistin can activate recombinant CDC42 but not Rho or Rac. Surprisingly, collybistin did not catalyze the exchange of GDP in the Rab GTPase identified in our screen. This may be due to the fact that the aminoacid sequence in the region responsible for the GTP hydrolysis of the Rab GTPase causes a very low intrinsic GTPase activity. However, we cannot rule out that collybistin might catalyze nucleotide exchange in this Rab GTPase in a cellular context. Our data suggest that collybistin can act as a specific activator for CDC42. Binding of the human homolog of collybistin to CDC42 has been demonstrated previously. Therefore, it is likely that the activation of CDC42 by a submembraneous complex composed of gephyrin and collybistin can lead to rearrangements of subsynaptic cytoskeletal elements which in turn might regulate synaptic morphology and function. The role of a possible interaction of collybistin with the newly identified Rab GTPase remains to be to be investigated. As Rab GTPases are required for vesicle transport, this interaction could be involved in trafficking and synaptic targeting of receptor containing transport vesicles.

756 A molecular role for gephyrin in the biosynthesis of molybdenum cofactor

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Gephyrin is a peripheral membrane protein that is essential for the aggregation of inhibitory glycine receptors at the postsynaptic membrane. A second function appears to be its role in the biosynthesis of molybdenum cofactor (Moco), the prosthetic group of molybdoenzymes, such as sulfite oxidase, xanthine oxidase, or aldehyde oxidase. In humans, the activities of these enzymes in liver and kidney are indispensable for life, since patients suffering from inherited moco deficiency develop incurable epileptic seizures and die in their early childhood. Remarkably, new-born gephyrin-deficient mice display similar neurological abnormalities, while they are also lacking molybdoenzyme activities and glycine receptors clusters at their synapses. According to this dual function, gephyrin seems to act as a scaffolding protein in different cell types; i) by orga-

nising receptor proteins at the postsynaptic membrane of neurons, and ii) by organising moco biosynthesis in epithelial cells, probably in a distinct subcellular compartment. -

Moco biosynthesis is highly conserved during evolution. In archaea, eubacteria, and eukaryotes, the biosynthetic pathway commences with GTP, which is converted to the precursor Z by the heterodimeric enzyme MOCS-1A/B. Another enzyme, MOCS-2A/B, then catalyses the conversion of precursor Z to molybdopterin, into which one molybdenum ion is finally inserted. Complementation studies in bacteria, plants, and eukaryotic cells have shown that gephyrin promotes this final step. -

Due to the chemical instability of its metabolites, moco biosynthesis is likely to proceed in a multienzyme complex that includes MOCS-1, MOCS-2, and gephyrin. To test this notion in a cellular context, we transfected HEK 293 cells with gephyrin and different sets of MOCS subunits, and examined their distribution by confocal immunofluorescence microscopy. Moreover, we performed co-immunoprecipitation studies of transfected cell extracts. So far, we have no evidence for a direct interaction between gephyrin and MOCS-1 or gephyrin and MOCS-2. Since additional factors may be required to assemble the predicted protein complex, molecules that may be missing from HEK 293 cells, we established a procedure to enrich for gephyrin-positive extracts from liver tissue and cultured hepatoma cells. We then generated polyclonal antibodies against all four MOCS subunits and immuno-purified the resulting antisera. Once these antibodies are fully characterised, we will use them to determine whether MOCS subunits co-purify or co-immunoprecipitate with gephyrin and whether these proteins co-localise in similar subcellular compartments. These results shall increase our understanding of the cellular organisation of moco biosynthesis in mammals, and shed light on whether moco biosynthesis shares organising principles with synaptogenesis.

Characterization of an antibody against collybistin, a guanine nucleotide exchange factor interacting with gephyrin: A possible role in glycine receptor clustering and function? 757

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Collybistin belongs to the family of guanine nucleotide exchange factors (GEFs) with a typical tandem dbl-homology (DH)- and pleckstrin-homology (PH)-domain. The DH-domain catalyses the GDP/GTP exchange reaction, whereas the PH-domain binds to phosphorylated phosphoinosides and thus mediates anchoring to the cell membrane. Collybistin was shown to bind to and activate monomeric GTPases of the Rho-family. Two splice variants of collybistin have been identified. Collybistin I has a

N-terminal SH3 domain and a C-terminal coiled coil domain, which are both missing in collybistin II. Collybistin was originally identified as binding partner for glycine receptor (GlyR) anchoring protein gephyrin. Coexpression experiments in HEK293 cells showed, that collybistin regulates the membrane deposition of gephyrin, which is known to be essential for the formation of GlyR-clusters at inhibitory synapses. In-situ hybridization with collybistin specific oligonucleotides revealed a high expression of collybi-

stin mRNAs in neurons. However, no data on collybistin expression and subcellular distribution of this protein is available. In order to study the expression as well as the subcellular localization of collybistin in neurons during synaptogenesis we have generated a collybistin specific antibody.

The characterization of collybistin with immunoblot analysis of transfected HEK293 using this antibody confirm the specificity for both splice variants. Using extracts from spinal cord and total brain of adult rats a major band can be detected, suggesting that collybistin is present in these tissues.

Currently we are establishing protocols for the analysis of collybistin expression by immunocyto- and histochemistry. This antibody will represent a useful tool to study investigate the subcellular distribution of this GEF during neuronal differentiation and development and will thus allow to study the functional role of collybistin for the formation of postsynaptic GlyR-clusters.

758 Structure function analysis and molecular interaction of the cysteine string protein of *Drosophila melanogaster*

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The highly conserved cysteine string protein of *Drosophila melanogaster* is found predominantly on synaptic vesicles, but also in other tissues such as follicle cells, testicles or spermatheca; low levels of the protein seem to be present in all tissues. CSP null mutants are semi-lethal and show reversible temperature-sensitive paralysis at 37°C; electrophysiological recordings at the NMJs reveal a complete loss of synaptic transmission at 32°C. Four distinct domains of the protein have been described so far: The J-domain (which shows high homology to bacterial DnaJ-proteins), the linker domain, the cysteine string (with the motif $C_2X_5C_{11}X_2C_2$) and the less conserved C-terminal domain. CSP interacts with Hsc70, syntaxin1A, G β subunits, synaptotagmin, SGT, PKA and CFTR. Speculations on CSP function in synaptic transmission include a role of the protein in the sensitization of presynaptic Ca^{2+}-channels or the enhancement of Ca^{2+}-sensitivity of a direct step in exocytosis. Common to all models is a co-chaperone function in conjunction with Hsc70. In order to elucidate the influence of the CSP domains on the biochemical properties of CSP, its subcellular distribution and its function in synaptic transmission we created transgenic flies expressing modified versions of the protein lacking the C-terminus, the linker domain or the cysteine string in a *csp* null-mutant background. The proteins lacking 27 amino acids at the C-terminus or 8 amino acids in the linker region show wild-type-like distribution in adults, whereas the deletion of the cysteine string eliminates the targeting of the protein to presynaptic terminals. Larvae expressing the constructs with a C-terminal or linker deletion display a block of synaptic transmission at 32°C , whereas the corresponding transgenic adults do not

paralyse at this temperature. We show that CSP interacts with G β also in *Drosophila* and that the C-terminal domain is not required for interaction with syntaxin (and possibly synaptotagmin). The unexpected differential effects of the transgenic proteins on larval and adult synaptic function make it necessary to study synaptic protein interactions also during development.

Molecular and phenotypical characterization of the *Drosophila synapsin* mutant

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Synapsins constitute a family of highly conserved and abundant phosphoproteins, identified almost three decades ago (Johnson et al., 1972). They seem to be involved in synaptogenesis as well as in the regulation of neurotransmitter release by controlling the availability of vesicles for exocytosis. In vertebrates there are three different genes, *synapsin* I, II and III, from which several isoforms are generated via alternative splicing. Mice lacking *syn* I, II or both genes develop normally and are fertile and viable. In knock–out mice lacking *syn* I paired pulse facilitation and recovery time after synapic depletion are increased, in *syn* II knock-out mice the post tetanic potentiation is decreased. In *Drosophila melanogaster* only a single *synapsin* gene is found. By P-element remobilization we generated a null mutant (w;;Syn^{97}) with a deletion of 1.4 kb eliminating the promotor region and the first exon. These mutants are viable and fertile and show no detectable morphological or ultrastructural phenotype. Electropysiological recordings failed to reveal significant defects in synaptic transmission. Complex behavior, however, is known to be strongly influenced by the white (w) genetic background of the mutant. To overcome this problem, we extensively outcrossed the synapsin mutant against the wildtype strain (CS). With the newly established Syn^{97CS} line we were able to uncover differences between wild type and mutant in several behavioral paradigms. For some of the experiments we used siblings from heterozygous crosses and subsequently determined the genotype by single fly PCR, thereby ruling out experimental bias and possible influences of the genetic background. Synapsin null mutants show a 9% increase in wing beat frequency compared to wild type flies. In the olfactory learning paradigm of Tully and Quinn the mutant displays a 25% decrease in the performance index, whereas olfactory perception seems to be normal. In the heat box learning of Wustmann and Heisenberg, where flies are trained to avoid a punished half of a small chamber, only male flies show a 50% reduction ($p < 0,05$) in the performance index in the test phase. Impairment in learning can already be detected in larvae (see contribution by Gerber et al.). At the molecular level we found evidence for an RNA editing modifying a codon in the protein kinase A recognition site in the “A” domain of the protein. This site is highly conserved in all known synapsins. In vertebrates, phosphorylation at this site is thought to regulate neurite outgrowth as well as dispersion and reclustering of the protein (Chi et al.,2001; Kao et al.,2002). We are presently studying this editing event during developmental stages as well as in several wild type lines including some stocks newly established from the wild. In the future we are planning to

take a closer look at the biochemical and physiological effects of RNA editing in synapsin by a kinase assay and further behavioral analysis.

Chi et al., (2001),12,1187,Nature Neuroscience Johnson et al. (1972),247,5650,J.Biol.Chem. Kao et al., (2002),5,431,Nature Neuroscience
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760 Differential alteration of the dystrophin-associated protein complex in brain and kidney of mice lacking utrophin or dystrophin

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Dystrophin and its autosomal homologue utrophin are membrane-associated cytoskeletal proteins that are part of a multimeric membrane-spanning protein complex, the dystrophin-associated protein complex (DPC). In muscle cells, the DPC comprises α- and β-dystroglycan and isoforms of syntrophin and dystrobrevin that are differentially associated with dystrophin or utrophin. The DPC is required for structural stabilization of the plasma membrane and for formation and maintenance of the neuromuscular junction. Utrophin and dystrophin are also expressed in other tissues, notably brain, choroid plexus, and kidney, but their functional role in these tissues is unknown.

The aim of this study was to determine whether utrophin and dystrophin are required for the formation and membrane-anchoring of the DPC in non-muscle tissue. Using high-resolution multiple immunofluorescence microscopy, we show here that dystrophin and utrophin form distinct DPCs in both brain and kidney of wildtype mice. In particular, a prominent utrophin-associated DPC was present in the basolateral membrane of kidney tubular epithelial cells. This DPC was disrupted in utrophin$^{0/0}$ mice, although it was partially replaced by a dystrophin-associated DPC, suggesting a compensatory role for dystrophin in the absence of utrophin. In contrast, in choroid plexus and in the endothelium of brain blood vessels, only an utrophin-associated DPC was observed, which was dramatically altered in utrophin$^{0/0}$ mice, without any evidence for compensation by dystrophin. These findings reveal that utrophin is necessary for the formation of a DPC. To test whether the same corresponds also for dystrophin, we investigated the distribution of DPC proteins in glial end-feet surrounding brain capillaries of mice lacking any dystrophin isoforms (mdx^{3cv}). In these mice, expression of DPC proteins in glial end-feet was profoundly reduced, whereas utrophin and its associated proteins exhibit normal distribution in endothelium and choroid plexus.

These results reveal a critical role for both utrophin and dystrophin in the formation of a DPC in various non-muscle cell types. Furthermore, a functional replacement of utrophin by dystrophin only occurs when the two proteins are co-expressed in the same cells in wildtype mice. It is therefore likely that the structure and/or function of the choroid plexus and the blood brain barrier is altered in utrophin$^{0/0}$ and mdx^{3cv} mice.

Identification and immunocytochemical lokalisation of tachykinin-related peptide and orcokinin-like peptides in the stomatogastric nervous system in three different decapod crustacean species. 761

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In this study we combined wholemount immunocytochemistry and mass spectrometry to investigate the distribution of a tachykinin–related peptide (TRP) and orcokinin-like peptides in the stomatogastric nervous system (STNS). TRP is one of the best investigated peptide families in the STNS of the crab *Cancer borealis* (Nusbaum et al., 2001), but has not been investigated in the crayfish *Cherax destructor* and in the lobster *Homarus gammarus* . In both species TRP-like immunoreactivity was present in all ganglia of the STNS, some of the connecting nerves and the pericardial organ. In the paired circumoesophageal ganglia (CoG) of *C. destructor* , two groups of somata, neuropil and numerous axons show TRP-like immunoreactivity. Both loosely and densely stained neuropil was found - the densely stained one is similarly shaped to the club-shaped structure found in *C. borealis* (Goldberg et al., 1988). Backfill experiments show that the axons of one stained group in each CoG, the ones close to the entrance of the superior oesophageal nerve, project in tight bundles into the STG where they terminate. Similarly, the axons of one large cell body in each CoG project to the STG. In *H. gammarus* , the staining is similar to the one found in *C. destructor* , with one major difference. There are two areas of densely stained neuropil: one club-shaped structure and one oval structure. The oval structure is particularly interesting since it seems to be the neuropil area into which the axons of the posterior stomach nerve (psn) terminate. This nerve contains the axons of the well investigated posterior stomach receptor (PSR) neurons. The staining pattern in *H. gammarus* is identical to the one found in *H. americanus* . In both *C. destructor* and *H. americanus* MALDI-TOF MS (matrix-assisted laser desorption time-of-flight mass spectrometry) post source decay fragmentation showed the presence of CabTRP Ia, previously identified in *C. borealis* (Christie et al., 1997). Since the neuropil area into which the psn projects is of special interest, we investigated that area using MALDI-TOF MS. We not only detected the masses of CabTRP Ia, but also of all five orcokinins, previously identified in *H. americanus* (Li et al., 2002). Our studies indicate that neuropeptides are not only present in single neurons, but also in clusters of somata. Furthermore, we have found candidates for transmitters of the PSR neurons, which will permit on investigation of the function of these peptides within the STNS in different decapod species.

762 Functional characterization of neurexophilins in the CNS

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Neurexophilins (Nphs 1-4) form a family of small molecular weight proteins that were initially discovered based on their strong binding to the synaptic membrane proteins α-neurexins. Nphs are secreted glycoproteins with structural features that resemble neuropeptides suggesting a possible signaling function mediated by α-neurexins. We have recently shown that α-neurexins are essential for normal synaptic neurotransmission apparently by controlling voltage-gated Ca^{2+} channel activity. Here, we provide a functional characterization of their extracellular ligands neurexophilin 1 and 3, the only isoforms in the rodent brain that bind to α-neurexins. A neurogenetic approach was followed generating three different mouse lines: First, a Nph3 knock-in (Nph3-KI) mouse line was established expressing a bicistronic mRNA encoding a fusion Nph3-Flag protein and β-galactosidase fused to a nuclear localization signal. Second, a Nph3 knock-out (Nph3-KO) mouse line was generated making use of the Cre-loxP mediated recombination, in which the Cre combinase was delivered by a transgenic mouse line. Finally, the double knockout of Nph1 and Nph3 (Nph1/Nph3-DKO) was obtained by crossing Nph3-KO with a previously established Nph1-KO line. Analysis of Nph3 expression in Nph3-KI mice was performed by assaying serial brain sections for β-galactosidase activity based on the co-expression of β-galactosidase in cells translating the endogenous Nph3. This method revealed the Nph3 expression pattern in the mouse brain with high accuracy and sensitivity suggesting a novel approach for the detailed *in vivo* identification of cells that express a particular protein: Nph3 expression was found to be highly localized with the most characteristic area of expression being in the deep layers of the neocortex (especially layer VIc), where a band of positive cells persisted throughout ontogeny and adulthood. In addition, transient occurrence of Nph3 was observed in a number of neuronal cell populations during development, for example, in the marginal zone of the developing neocortex and in the dentate gyrus of the hippocampal formation, both of which could be identified as Cajal-Retzius cells by double-labeling against reelin. Preliminary analysis of the single knockouts as well as the Nph1/Nph3-DKO mice showed a slightly reduced viability for Nph1, but no gross anatomical abnormalities in brain structure. Currently, behavioural tests and *in vitro* experiments using recombinant Nphs are under way to further elucidate the function of these elusive molecules. We speculate that different Nphs may be used by distinct neuronal populations, where they act as locally expressed modulators of the α-neurexin function at synapses.

Effects of hypothalamic neuropeptides on dopaminergic and GABAergic neurons in the ventral tegmental area (VTA) of the rat. 763

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Ventral tegmental area (VTA) dopaminergic and GABAergic neurons are critically involved in brain mechanisms of reward, reinforcement and emotional arousal. The firing of dopamine neurons in this region is closely correlated with the availability of primary rewards, such as food. The VTA receives a massive input from the lateral hypothalamic area, including projections from neurons containing orexins - neuropeptides that enhance both food intake and arousal. We found recently that orexins increase the firing rate and depolarize the majority of dopaminergic and GABAergic VTA neurons (J.Neurosci, 2003; 23(1):7-11). They also cause oscillatory responses, accompanied by burst firing, in a subgroup of dopaminergic cells *in vitro*. In this study we investigated the effect of other feeding- and arousal-related neuropeptides on the firing rate and membrane potential of VTA cells by extracellular and whole-cell recordings *in vitro*. The expression of these peptides and their receptors was studied in identified single neurons by RT-PCR. Brain slices were prepared from 3-4 week-old male Wistar rats. We found that neuropeptide Y (NPY, 300nM, higher doses in unresponsive cells were also tested), the most potent orexigenic peptide known, inhibited the firing of 40 % of dopaminergic cells, two of them completely, others up to 65%. All NPY-inhibited neurons were excited by orexins. NPY inhibited 50% of GABAergic neurons tested. An anorectic hypothalamic neuropeptide α-melanocyte stimulating hormone (α-MSH) had no effect on DAergic cells (up to 1μM), but excited several GABAergic cells which were inhibited by NPY. Corticotropin-releasing factor (CRF), an anorectic peptide which is also known to promote arousal, excited 60% of dopaminergic neurons; the increase in firing rate was accompanied by depolarization (4 mV); CRF also increased the firing rate of GABAergic neurons. Neither the orexigenic neuropeptide ghrelin nor the anorectic peptide cocaine and amphetamine-related transcript (CART) affected the firing rate or membrane potential of VTA neurons. Substance P (SP) that promotes arousal but not food intake, excited the majority of VTA neurons (300nM). Single-cell RT-PCR experiments on acutely-isolated, identified VTA cells demonstrated the presence of mRNA for the peptides tested or for their receptors. Our results suggest that there is no clear correlation between the ability of hypothalamic peptides to stimulate food intake and their ability to excite or inhibit dopaminergic neurons. In contrast, peptides which increase arousal all seem to excite VTA neurons. This study also emphasizes the unique action of orexins on dopaminergic system: they are the only neuropeptides, which cause burst firing in dopaminergic neurons *in vitro*.

765 Activity-dependent suppression of spontaneous spike generation in the Retzius neurons of the leech, *Hirudo medicinalis*

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Spontaneous spiking as well as sustained periods of spike suppression following enhanced firing activity (post-stimulus-depression, PSD) have been demonstrated in serotonin (5-HT)-containing neurons in both vertebrate and invertebrate species [Heinrich et al. 1999, *Proc Natl Acad Sci* 96, pp. 2473-2478; Wang & Aghajanian 1977, *Brain Res* 132, pp. 186-193]. It has been hypothesized that PSD may be a unique feature of amine-containing neurons throughout the animal kingdom.

To further investigate spiking patterns and mechanisms of rhythmogenesis, we conducted intracellular *in-vivo* recordings (current-clamp) from identified neurons within a chain of usually 5 intact ganglia dissected from the ventral nerve cord of the medicinal leech, *Hirudo medicinalis*. We used a standard stimulus protocol consisting of depolarizing current-pulses of different amplitude (0.1 - 2 nA; 5 s) and duration (5 - 20 s; 1 nA) leading to spiking frequencies of up to 15Hz. In addition to inducing spike trains by tonic current injection, we also used spike-event controlled phasic stimulation to elicit precisely timed single action potentials (APs).

As reported elsewhere [Dean & Leake 1988, *Comp Biochem Physiol* 89C, pp. 31-38], the 5-HT containing Retzius (R) cells spontaneously generate a regular spiking pattern with a firing frequency ranging between 0.1 and 1.5 Hz (mean: 0.5 ± 0.28 Hz) as was also confirmed by analysis of the spike interval distribution (variation coefficient C_V: 0.3 ± 0.11). Current-induced high frequency spiking was characteristically followed by a period of sustained spike depression. During PSD, the membrane potential remained slightly hyperpolarized and gradually returned to the pre-stimulus resting potential in parallel with the spiking activity. Quantitative analysis showed that the duration of PSD (range: 2.1 - 22.7 s) is positively correlated with (i) the magnitude and (ii) the duration of intracellular current injection. Moreover, a significant inverse correlation exists between the initial spiking frequency and the duration of the PSD.

To analyse possible mechanisms underlying the observed PSD, experiments were carried out in synaptically isolated R-cells in ganglia superfused with high Mg^{2+} (20 mM). Under this condition PSD was still prominent. Thus, 'auto-inhibitory pathways' driven either by an increased and activity-dependent release of endogenous 5HT by the R-cells themselves - thus mediating self-inhibition - or activation of inhibitory networks feeding back to the R-cells via interneurons can most likely be ruled out.

Therefore, we started to investigate possible intrinsic membrane properties using single evoked APs. We found, that single evoked spikes in high [Mg^{2+}] lead to a significantly prolonged post-stimulus spike interval and to a phase shift in the following spike intervals. However, the observed phase-shift only occurred when the 'additional' spike was elicited shortly (i.e. less than ~20% of the phase duty cycle) after a preceding AP. These data, in conjunction with controls using subthreshold stimuli failing to induce compa-

rable effects, indicate that PSD might be coupled to the intrinsic AP-generating mechanism of the investigated neurons.

Since pronounced PSD, although present in all amine-containing neurons tested so far, including the octopaminergic Leydig cells, has also been found in non-aminergic spontaneously active neurons (AP-cells, AE-motoneuron), we conclude that PSD cannot be regarded as an exclusive feature of aminergic neurons in the leech CNS.

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A central nervous role for proctolin: Control of singing behavior by activation of the adenylate cyclase- and phospholipase C-pathway in the brain of grasshoppers

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The pentapeptide proctolin is known as a neurotransmitter and modulator of neural and muscle activity in insects, crustaceans and annelids. Its main physiological role, is that of a modulator, being released as a co-transmitter at different effectors such as visceral muscles, gut, heart, antennal "heart" and in rhythm generating circuits (1). Proctolin-like immunoreactive cell bodies have been found in the brains of various insects. In locusts three pairs of medium sized cells in the protocerebrum(2) and 30-50 smaller cell bodies in the pars intercerebralis that innervate two distinct layers of the fan shaped body of the central complex (3) have been described. While little is known about central nervous functions of proctolin, studies with various analogs suggested the existence of at least two subtypes of proctolin receptors (4). These are G protein-coupled receptors that appear to act through the phospholipase C transduction pathway leading to rising intracellular Ca^{2+} concentration.

Our pharmacological studies with intact behaving grasshoppers suggested that proctolin, in addition to muscarinic receptor activation, may also contribute to the selection and control of specific acoustic communication behaviors. The species-specific songs can be stimulated in a dose-dependent fashion by pressure injection of proctolin into the central body complex and a small neuropil anterior to it. Most of these stimulation sites coincide with sites where muscarinic stimulation also elicits stridulation, although at few stimulation sites only one of the two neuroactive substances was effective, suggesting that the respective receptors might be situated on different neurons. At sites, where both proctolin and muscarine elicited stridulation, proctolin-stimulated behavior occurred after shorter latencies and was of shorter overall duration.

Proctolin-stimulated stridulation can be suppressed by inhibition of both adenylate cyclase activity with SQ 22536 and also by inhibition of phospholipase C activity with neomycin. Similarly, activation of both the adenylate cyclase and the phospholipase C signaling pathway has previously been demonstrated to follow muscarinic receptor activation in the cephalic control system for communication behavior (5).

Proctolin as well as acetylcholine should be released from projection neurons into the stridulation control circuit upon appropriate sensory stimulation, leading to increased basal excitation mediated by the above mentioned intracellular signaling pathways which promotes the initiation of stridulation.

(1) Orchard et al. 1989, J Neurobiol 20: 470-496;
(2) Bishop & O'Shea 1982, J Comp Neurol 207:223-238;
(3) J. Issberger, PhD thesis, University of the West of England;
(4) Lange & Orchard 1993, J Insect Physiol 39: 347-351;
(5) Wenzel et al. 2002, J Neurophysiol 87: 876-888

767 Daily structural changes of dense core vesicles in PDH-ir neurons in the optic lobe of the housefly's brain

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The optic lobe of *Drosophila melanogaster* contains a cluster of small and large ventral lateral neurons (LN_vs). These so-called "clock" neurons express clock genes and show colocalized a peptide pigment dispersing factor (PDF) closely related to crustacean pigment dispersing hormon (PDH). PDF has been suggested to be a transmitter in the neuronal circadian system of *Drosophila*, and contribute to synchronize circadian pacemaker neurons in both brain hemispheres.

In the housefly *Musca domestica*, four neurons immunoreactive to the pigment-dispersing hormone (PDH-ir) show similarity to *Drosophila's* large LN_vs. They form a distinct layered network of varicose fibres in the lamina and medulla neuropiles of both ipsi- and contralateral optic lobes. The varicosities of these PDH-ir neurons exhibit circadian modulation in their sizes and spacing, demonstrated previously by confocal microscopy (I.A.Meinertzhagen; E. Pyza, 1997, Eur. J. Neurosci., 9: 1784-1788).

We studied the PDH-ir neurons in the distal medulla of the housefly brain, using immuno light and electron microscopy, in particular the ultrastructure of PDH-ir varicosities, its organells, and structural changes. Moreover, stimulation experiments with brains were performed to investigate mechanisms of PDH-release. For the immunocytochemical experiments, flies were reared under controlled temperature and light-dark conditions (LD 12:12), and decapitated one hour after light-on (ZT1) or one hour after light-off (ZT13).

PDH-ir varicosities at the distal rim of the medulla are filled with dense core vesicles (DCVs) containing PDH as shown by postembedding immuno electron microscopy. Varicosity profiles with PDH-ir DCVs outnumber other DCV containing varicosities of unidentified, immuno-negative profiles. Conventional electron microscopy seldom showed both DCV accumulations and synaptic specializations of the fly type in unidentified fibre profiles. Exocytotic ω figures of DCV membrane fusion are rarely encountered, appearing always apart from synaptic sites. This may points to a non-synaptic paracrine mode of release of contents from DCVs, including PDH-ir varicosity profiles. The latter seems to lack synaptic input and output sites.

The amount of DCVs in PDH-ir profiles is significantly lower in ZT1 than in ZT13 (DCV numbers/area ratio 3:4). During the night, the PDH-DCVs are mainly found translucent or weakly electron dense, whereas their electron density is apparently enhanced during the day. These day correlated morphological changes are suggested to indicate circadian fluctuations of PDH-DCV contents. Not finding synaptic links of LN_v varicosities in the medulla to other neuronal circuits, we tested the effect of depolarizing stimuli to isolated brains, using bath application of highly concentrated potassium Ringer solutions. We found PDH-ir experimentally reduced versus control brains. The release of PDH from DCVs could therefore be triggered by bioelectric events.

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Cellular Parameters Modulated by Octopamine in Context of a Switch-On of Recurrent Action in Auditory Lateral Inhibition of Crickets 768

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The thoroughly investigated auditory pathway in the prothoracic ganglion of Gryllus bimaculatus comprises a recurrently coupled pair of lateral inhibitory nerve cells ON1 (Hirtz, Wiese J comp Neurol 386:347ff (1997). Only in the presence of octopamine released from Dorsal Unpaired Median cells (Hoerner J Micr. Res. 44:137ff 1999) the circuit of two cells develops the properties for which recurrent inhibitory systems are famous: Low common mode suppression, High differential mode gain and Frequency dependency of circuit output. Of course all three factors depend on the strength of synaptic coupling established between the two cells (Eilts, Wiese Biol Cybern 51:45ff 1984). The exemplary simplicity of the circuit consisting of only two cells invites an attempt to identify the cellular parameters affected and effects generated by the modulator. In the extensor tibiae muscle of the locust (Walther, Zittlau J Neurophysiol.80: 785ff.1998) octopamine evoked hyperpolarization-activated outward directed chloride current and a decreased transmembrane conductivity for potassium ions. This information has lead us to hypothesize, that an increased length constant in the ON1-axon could transport even graded potentials like postinhibitory rebound depolarisations all along the ON1 axon back to the side of the stronger stimulated input. Secondly, octopamine is also reported to increase synaptic efficacy, a fact which regularly increases the amplitude of postinhibitory rebound depolarisations. Gross asymmetries between sensitivities of ON1 neurons right and left, *in vivo* induced by asymmetric foreleg posture (Hill, Boyan J comp Physiol 1977) alternatively induced by asymmetric octopamine-modulation of one of the two ON1 neurons, may be an additional prerequisite that a respective recurrent cell under these conditions becomes active.

As a starter of our investigation and to align suitable methods and reasoning for investigation of the situation in ON1, we measured octopamine effects on the transmembrane resistance in locust flight muscles. Rating from the increased voltage response to constant pulses of injected current without and with octopamine 10^{-5} M bath applied, we

found that octopamine raises in some of the impaled muscles the transmembrane resistance by a factor of 2. Interestingly the effect of octopamine was suppressed by 2mM Ba2+, known to block or at least affect the control of some potassium channels. However, the transmembrane resistance did not change in response to the treatment with Ba2+ ions jointly with 10^{-5} M octopamine as compared to the normal situation. The octopamine effect on transmembrane resistance is combined with a slight increase in resting potential, a hint (U. Bickmeyer, pers. comm.) that octopamine may reduce an I_h current, a mixed current activated by hyperpolarizations (Pape HC Ann Rev Physiol 58:299ff 1996). Single electrode voltage clamp measurements are to identify the respective species of ions which helps to increase transmembrane resistance first in muscle cells then in ON1 neurons. Equipment by DFG: Wi 363/19-1

769 Regulation of the endocannabinoid systems by dietary oils as possible therapy for treating weight loss associated with eating disorders

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The endocannabinoid (EC) system has recently been implicated in the regulation of feeding and cognitive function. Since ECs are derived from essential fatty acids, dietary manipulation may lead to new strategies for the treatment eating disorders.

Aims: To evaluate the effect of low dose endocannabinoids in mice under diet restriction and to manipulate, using their precursors from dietary oils, 2AG levels in the hypothalamus (feeding regulation), hippocampus (cognitive function) and whole brain.

Methods: A: Young Female Sabra mice were fed on 2.5 hours a day diet for 14 days. During the second week they were administered with 0.001mg/kg/day anandamide, 2AG or noladin (arachidonate ether) with and without antagonist. Food consumption and cognitive function were evaluated. B: Female Sabra mice were fed diet restriction (DR) for 12 days. DR 100% (weight maintenance), DR 60% and DR 40%. The DR 100% diet was supplemented with 20% soya, or 5% soya, olive or coconut oil or 2.5%soya oil. Mice were sacrificed after 12 days and whole brain hypothalamus and hippocampus were analyzed for 2AG levels using TLC and GC-MS.

Results: DR led to a diet-related decrease in 2AG levels in all brain areas. Low doses of endocannabinoids (0.001 mg/kg.ANA11, 2-AG and noladin) improved food consumption, cognitive function and reversed some of the neurotransmitter changes following diet restriction (DR). These effects were blocked by antagonists of the cannabinoid receptor 1 (CB1). Supplementation of soya oil decreased it further, while coconut (saturated) or olive (monounsaturated) oils did not produce significant change. However, 2.5% soya oil showed an increase in 2AG levels.

Discussion: If applicable to human subjects, soya oil supplementation might enhance appetite in patients with anorexia nervosa.

Nitric oxide and angiotensin II - neuromodulators in thermoregulation during exposure to combined heat and hypohydration stress

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In mammals, perturbation in body fluid homeostasis interferes with the ability to cope with thermal stress, as the need for water conservation overrides thermoregulation. Most of the current knowledge regarding the hypothalamus, as the main homeostasis-controller site is limited to phenomenology, displayed by thermoregulatory integrative functions. Based on the individual involvement of central Renin-Angiotensin system (RAS) and Nitric Oxide (NO), in fluid balance and adjustment of autonomic heat defense, we aimed to evaluate the involvement of NO in the integration between osmo and thermoregulatory circuits, and to define the mutual effect of NO and AngII. For this purpose, we used rats (Rattus Norvegicus, Sabra strain, albino var.) either euhydrated or hypohydrated (-10% of the body weight). 7-Nitroindazole (100nm in a bolus) – an antagonist of neuronal Nitric Oxide synthase, AngII (100pm), or both, were administered to the cerebrolateral ventricle. Data were collected using three different experimental paradigms: 1) Heat defense responses -vasodilatation, evaporative cooling (salivation threshold), and endurance time measured in conscious heat stressed (39°C) rats. 2) The encoded AngII receptors (AT1, AT2) and nNOS, essential components in RAS and NO signaling were measured in hypothalamic homogenates using western imunoblotting 3) Transcription levels of these genes were measured using quantitative real-time PCR.

Our results illustrate the influence of the agents used in all studied parameters. Ni has a greater impact in the hypohydrated state than in the euhydrated state. Administration of the drugs individually did not always have the same impact as administration of both together, implying a mutual effect. AT1 receptors revealed greater sensitivity to AngII and Ni administration than AT2. AT2 levels were stable, thus marked changes in AT1/AT2 ratio in the various physiological situations were noted. Changes in transcription levels were more pronounced than in protein levels.

We conclude that NO plays an important role in neuromodulation of thermoregulation primarily via modulating AngII receptors, particularly in the hypohydrated state. A novel finding in this investigation is the involvement of AT2 receptors in thermoregulation, particularly via altering AT1/AT2 receptor density ratio. Diverse responses to the same pharmacological manipulation in different hydration states are seen, implying that different pathways are activated as a consequence of water shortage or heat stress.

771 Signal Transduction Mechanisms involved in the potentiation of muscle contraction by the neuropeptide Proctolin

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The neuropeptide proctolin was localised immunochemically in a single pair of motoneurones in the marine isopod *Idotea emarginata*. In the extensor muscles, 10^{-6} M proctolin potentiates muscle contractions through synergistic pre- and postsynaptic mechanisms. Postsynaptically, it phosphorylates a regulatory muscle protein associated with the actin filament (Brüstle et al., 2001), it increases the input resistance of muscle fibres by closing voltage-independent K^+ channels and it increases the inward current through L-type Ca^{2+} channels. The latter effects were mimicked by increasing the intracellular cAMP-concentration. (for summary of effects see Rathmayer et al., 2002)

The aim of the present study was to investigate proctolin-induced intracellular signalling pathways in the extensor muscle fibres. Measuring intracellular concentration of cyclic nucleotides showed that the cAMP-concentration was not increased after applying proctolin (10^{-9} M, 10^{-6} M) to the muscle fibres. However, proctolin induced a significant decrease of the intracellular cGMP-concentration. This decrease of cGMP-concentration is mediated via a PKC and is independent of the activation of cGMP-specific phosphodiesterases.

In single fibre preparations which allow the recording of contractures and simultaneously the analysis of membrane potentials, proctolin also potentiates contractures elicited by high (30 mM) K^+ up to 47% without changing the membrane potential. Blocking the PKC with a cell permeable specific-PKC inhibitor prevented the potentiation of potassium-contractures by proctolin. The potentiation was also prevented by counteracting the proctolin-induced decrease of cGMP after applying a phosphordiesterase-resistant cGMP analog.

These results suggest that the potentiation of contractions by proctolin might also involve an activation of PKC.

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772 Nitric oxide as an endogenous modulator of circadian pacemaker cells in the snail *Bulla gouldiana*

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The marine mollusc *Bulla gouldiana* is now an established animal model in circadian biology (Block and Michel 1993. Int. Rev. Cytol. 146:83). We now present evidence suggesting that nitric oxide (NO) is a key efferent modulator of circadian pacemaker cells in this system.

Putative NO-releasing structures were revealed in the neuropil of the eye surrounding the somata of the so-called basal retinal neurons (BRNs), which are circadian pacemaker cells. The labeled profiles appear to arise from extrinsic neurons with axons entering the eye via the optic nerve. Basal retinal neurons in primary cell culture were loaded with the fluorescent calcium probe fura-2 to investigate the effect of NO-cGMP signaling pathway related substances on intracellular calcium concentration. Bath application of the NO donors sodium nitroprusside (SNP) and the more specific S-nitroso-N-acetylpenicillamine (SNAP) lead to an elevation of the intracellular calcium concentration. This elevation is blocked by simultaneous application of ethylene diamine tetraacetic acid (EGTA), which buffers calcium ions to a low concentration, indicating that NO cause an influx of calcium ions presumably through cGMP gated channels. Supporting this idea, bath application of the phosphodiesterase blocker isobutylmethylxanthine (IBMX) also elevated the intracellular calcium concentration. This is most likely due IBMX unmasking the basal activity of an endogenous, cGMP producing, presumptive retinal isoform of guanylate cyclase, which is normally consumed by phosphodiesterases. Finally, extracellular recordings from the optic nerve show that 8-bromo-cGMP enhances the light induced responses of BRNs in the intact, dark-adapted eye. Contrasting this, N^{ω}-nitro-L-arginine methyl ester (L-NAME), a specific but irreversible inhibitor of NO synthesis, reduces the light induced responses. The effect of L-NAME can be compensated by subsequent application of 8-bromo-cGMP. We propose that NO released from neurons entering the eyes is likely to modulate the light responsiveness of the circadian pacemaker cells in Bulla by activating cGMP-gated calcium channels.

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Differential mRNA expression of kinin receptors and nitric oxide synthase isoforms in hypothalamus and brainstem during LPS-induced inflammation in rats

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Lipopolysaccharides (LPS), the pro-inflammatory substances present in the cell wall of gram-negative bacteria, either injected or generated during the course of gram-negative infections induces several central effects such as fever, inhibition of water and food intake, reduction in locomotory activity, anxiety and depressive-like episodes. It has been postulated that most central effects induced by LPS are probably mediated through kinins, prostaglandin and nitric oxide (NO). Bradykinin (BK) and its active metabolite des-Arg9-BK are pro-inflammatory mediators. They act through the activation of B2 and B1 receptors, respectively. These neuromodulatory peptides influence the central regulation of blood pressure, pain and core temperature. Most of these physiological functions are mediated through kinin B2 receptors present constitutively. The B1 receptors can be induced by LPS.

Using RT-PCR, we measured in adult male SD-rats the gene expression (at mRNA level) of kinin B1 and B2 receptors and the nitric oxide synthase (NOS) isoforms [neu-

ronal (n), endothelial (e) and induceable (i) NOS] in the hypothalamus and medulla oblongata 1, 3 and 6h after a single i.p. injection of LPS (5 mg/kg) and compared with vehicle treated controls.

There was a significant and time-dependent increase in the B1 and iNOS mRNA levels in the hypothalamus [consisting of nucleus paraventricularis (PVN) and supraopticus (SON) and part of the lateral hypothalamus] of LPS-treated rats. The highest mRNA levels were found at 6h following LPS. Whereas, no change was found in the mRNA levels of either B2 receptor, nNOS or eNOS. In the dorsal and ventral parts of the medulla containing nucleus tractus solitarius (NTS) and area postrema (AP), and rostral-ventro- (RVLM) and caudalventro lateral medulla (CVLM), respectively, there was a significant increase in the B1 receptor mRNA level following 6h LPS, whereas the iNOS mRNA level reached its maximum at 3h and declined at 6h after LPS. In these medullary regions, there was also no change either in the B2, nNOS or eNOS mRNA levels in LPS-treated rats. We also localize the neurons within the hypothalamus and brainstem regions which were activated following LPS i.p. by immunohistochemical staining of c-Fos expression, a transcriptional factor expressed immediately following activation of cell/neuronal . Interestingly, pretreatment of rats with Lys-(des Arg9, Leu8)-BK, a specific B1 receptor antagonist, given intracerebroventricularly (200 pmol/2 µl) completely abolished the LPS-induced c-Fos expression in the hypothalamic PVN and SON and in medullary regions such as NTS, AP, RVLM and CVLM. Whereas, pretreatment of rats with Hoe 140, a specific B2 receptor antagonist, did not completely abolished the LPS-induced c-Fos expression.

Our present molecular and immunohistochemical data provide evidence that the neuroendocrine effects induced by LPS given i.p. are mediated through the differential regulation of kinin B1 receptor and iNOS genes in the hypothalamus and medulla oblongata, and may imply an involvement in the pathophysiological effects of LPS-induced inflammation.

774 Hypothalamic neuropeptides are differentially expressed in rat models of obesity and type-2 like diabetes

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Background and objective: The hypothalamus plays a critical role in the regulation of energy balance. Several hypothalamic neuropeptides such as neuropeptide Y (NPY), prepro-orexin (PPO), melanin-concentrating hormone (MCH), agouti gene related peptide (AgRP), pro-opiomelonocortin (POMC), and cocaine- and amphetamine-regulated transcript (CART) are modulated by fasting and/or leptin and interact to regulate food intake and energy expenditure. Leptin resistance caused by a mutated leptin receptor results in obesity without apparent diabetes in Zucker fatty rats. In contrast, Zucker diabetic fatty (ZDF) rats, a substrain of Zucker fatty rats, develop type-2 like diabetes. To evaluate a possible role of hypothalamic peptides in the development of diabetes in ZDF rats we analyzed the gene expression of various peptides. We compared rats with

the genetic background of Zucker fatty and of ZDF rats either with or without mutated leptin receptors.

Methods: Hypothalami were dissected and total RNA was extracted by the guanidinium isothiocyanate method. After purification over silica-gel-based spin columns, the RNA was reverse transcribed to cDNA. Quantitative measurements of NPY, PPO MCH, AgRP, POMC, CART, and GAPDH cDNA was performed by kinetic real-time PCR on a GeneAmp 5700 sequence detection system using SYBR green I as fluorescent dye. The initial mRNA copy numbers in each sample were calculated by the cycle threshold method. Effects of leptin resistance and genetic background were estimated by two-way variance analysis (ANOVA) and subsequent group comparisons by the Bonferroni's post test.

Results: In obese Zucker fatty rats and diabetic ZDF rats, hypothalamic POMC and CART mRNA levels were reduced compared to the respective control rats with intact leptin receptors. In addition, CART mRNA levels were significantly higher in rats with the genetic background of ZDF rats compared to rats with the genetic background of Zucker fatty rats. POMC mRNA levels were not different between the two genetic backgrounds. In ZDF but not in Zucker fatty rats, NPY mRNA levels were increased compared to controls. No differences between PPO, AgRP, and MCH were found between obese or diabetic rats and their control or between rats with the Zucker fatty or ZDF background. Elevated plasma corticosterone levels indicated an activated hypothalamo-pituitary-adrenal axis rats with ZDF background.

Conclusion: Our results show that specific differences exist in the expression of hypothalamic peptides between Zucker fatty and ZDF rats. In combination with leptin resistance, higher mRNA levels of CART may be involved in the development of diabetes in ZDF rats.

Quantification of orexin receptor mRNA in distinct brain nuclei using quantitative real-time PCR

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Background: The hypothalamic peptides orexin A and orexin B are considered to be of importance in various basic physiological processes, such as sleep, feeding and energy homeostasis. Their two G protein-coupled receptors (OX_1 and OX_2) are expressed in many areas of the brain involved in the regulation of these processes. To exactly quantify the amount of orexin receptor mRNA in specific brain nuclei we have combined quantitative real-time PCR with the micro-punch technique.

Material and methods: The brains of male rats were removed and rapidly frozen. The brains were cut alternatingly into frontal 300 µm and 20 µm thick slices at -8 to -11°C. The 300 µm slices were dissected using a punch technique with the smallest removed brain regions measuring 520 µm, the largest 1,2 mm in diameter. Brain punches were collected directly into guanidinium isothiocyanate buffer and homogenized. Total RNA

was purified using silica-gel-based spin columns. After extraction, total RNA was reverse transcribed. cDNA was amplified using OX_1 and OX_2 receptor sequence specific primers by real-time PCR using SYBR green I as fluorescent dye. GAPDH was used as internal standard. As verification of the position of each punch, the 20 μm brain sections were Nissl stained and compared to the adjacent punched slices. The areas of interest were locus coeruleus (LC), nucleus paraventricularis (PVN), lateral hypothalamus (LH), taenia tecta (TT), raphe obscurus (RO), cortex, cerebellum, and the neuroepiphysis.

Results: In the LC we found the highest levels of OX_1 receptor mRNA (60-fold higher compared to OX_1 receptor in the cortex). TT, LH, RO, epiphysis and PVN contained moderate levels of OX_1 receptor mRNA, while the lowest amounts of OX_1 receptor mRNA were found in the cortex and the cerebellum. The levels of OX_2 receptor mRNA were more homogenous among the specified nuclei, the highest appearing in the PVN, the lowest in the epiphysis. In general, OX_1 receptor mRNA levels were higher than those of OX_2 receptor mRNA. The only exceptions were the PVN and the cerebellum, where OX_2 receptor mRNA levels were slightly higher expressed than OX_1 receptor mRNA.

Conclusion: Combining the micro-punch technique and quantitative reverse-transcription real-time PCR provides a powerful tool for the determination of exact amounts of mRNA levels in specific brain nuclei. We were able to quantify mRNA levels of OX_1 and OX_2 receptors in brain punches as small as 0.064 mm^3. Our data extend previous results showing a rather roughly estimated distribution of orexin receptors by semi-quantitative *in situ* hybridisation.

776 Involvement of a neuropeptide related to orcokinin in light entrainment of the circadian clock of the cockroach

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The daily course of behavioral and physiological events is regulated by endogenous circadian clocks in probably all organisms. The timing of locomotor activity of the cockroach *Leucophaea maderae* is orchestrated by two synchronized and bilaterally organized circadian pacemaker centers within the optic lobes. Accumulating evidence suggests that the accessory medulla (AMe) at the ventromedial edge of the medulla is the site of the circadian clock within the brain.

Four categories of neurons can be distinguished in the AMe: photic input elements, local interneurons, output elements, and, finally, two types of heterolateral coupling units. One type of coupling units, immunoreactive to pigment-dispersing hormone (PDH) and FMRFamide, invades the AMe and the distal surface of the medulla (Petri and Stengl 1997, J Neurosci 17:4097; Review: Helfrich Foerster et al 1998, Chronobiol Int. 15:567). The second has bilateral arborizations in the AMe and in several median layers of the medulla. Neurons of the second type are responsive to polarized light stimulation,

but not immunoreactive to PDH (Loesel and Homberg 2001, J Comp Neurol 439:193; Reischig and Stengl 2002, J Comp Neurol 443:388).

Asn^{13}-orcokinin is a myotropic peptide isolated from the abdominal nerve cord of the crayfish, *Orconectus limosus*. This peptide is a member of a highly conserved family of crustacean tridecapeptides (Stangier et al 1992, Peptides 13:859). To investigate the possible presence of orcokinin-related peptides in the cockroach, we used an antiserum against Asn^{13}-orcokinin. Twenty-four to thirty orcokinin-immunoreactive (-ir) somata were found in five of six anatomically distinguishable groups of the AMe (Reischig and Stengl 1996, Cell Tissue Res 285:305). Four of them have contralateral projections via the posterior optic commissure.

Double labelling experiments with antisera against orcokinin and PDH- or FMRFamide showed three somata with colocalized PDH/orcokinin-immunoreactivties and three to four somata with FMRFamide/orcokinin immunostaining. No colocalization, however, occurred in the anterior optic commissure, or in the posterior optic commissure, which are used by contralaterally projecting PDH- and FMRFamid-ir neurons. This suggests that the contralaterally projecting orcokinin-ir neurons do not belong to the PDH- or FMRFamide-ir neurons.

Microinjections of Asn^{13}-orcokinin into the medulla of the cockroach revealed phase delays at the late subjective day, similar to the effect of light pulses applied at this time. Therefore, we assume that orcokinin-ir neurons of the AMe play a role in the transmission of light- and coupling information to the contralateral pacemaker.

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Are FMRFamide-related peptides involved in the circadian coupling pathway of the cockroach *Leucophaea maderae*? 777

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The timing of locomotor activity of the cockroach *Leucophaea maderae* is orchestrated by synchronized circadian pacemaker centers in each accessory medulla (AMe) of the optic lobes (Reischig and Stengl, 2002, J Comp Neurol. 18:443). Synchronization of the bilaterally symmetric pacemakers is achieved by direct neuronal coupling pathways. A group of pigment-dispersing hormone-immunoreactive (PDH-ir) neurons arborizes in the AMe and connects both optic lobes via several anterior and posterior commissures (Reischig and Stengl, 2002, J Comp Neurol. 18:443). Six of these PDH-ir neurons are also FMRFamide-ir (Petri et al. 1995, Cell Tissue Res 282:3). FMRFamide-immunoreactivity suggests the presence of either a sulfakinin, a FMRFamide, a leucomyosuppressin (LMS) or a head peptide in the circadian clock. Because computer modeling predicted that the phase delaying PDH cannot be the only coupling signal synchronizing both pacemaker centers (Petri and Stengl, 2001 J Biol Rhythms 16:125), we tested whether any FMRFamide-related peptide might be the missing coupling signal causing phase advances.

With antibodies against LMS (XVFLRFamide, R. Nichols), FMRFamide (E. Marder), perisulfakinin (XGHMRFamide), and head peptide (XLRLRFamide, H. Agricola) we compared the arborization pattern within the AMe and in commissures connecting both AMae. FMRFamide- and perisulfakinin-immunoreactivity was found in commissures and neurons of the AMe. The perisulfakinin-ir somata were weaker stained than the FMRFamide-ir somata. Neither anti-head peptide- nor LMS-immunoreactivity was found in AMe neurons. Because we observed strong FMRFamide-, weaker XGHMRFamide- and neither XVFLRFamide- nor XLRLRFamide-immunoreactivity, we currently test whether XFMRFamides, but not XLRFamides phase shift circadian locomotor rhythms.

Injections of LMS into the vicinity of the AMe did not result in phase-dependent phase shifts of locomotor activity rhythms. But injections of FMRFamide into the pacemaker region appeared to cause phase delays, but with higher variability compared to PDH-dependent phase shifts. Thus, we are currently testing whether different members of the FMRFamide-related peptide family cause time-dependent phase delays and phase advances after injection into the AMe and might thus be non-photic, possibly coupling input pathways into the circadian clock. [Supported by DFG grants STE531/7-3 and Human Science Frontiers]

778 Extracellular long-term recordings of the accessory medulla, the circadian pacemaker of the cockroach *Leucophaea maderae*

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The temporal organization of physiological and behavioral states is controlled by circadian clocks in apparently all eukaryotic organisms. In the cockroach *Leucophaea maderae* transplantation studies (Reischig and Stengl 2003, J. Exp. Biol. submitted) located the circadian pacemaker in the accessory medulla (AMe). The AMe is densely innervated by peptidergic neurons, among them the pigment-dispersing hormone-immunoreactive (PDH-ir) neurons, some of which are also FMRFamide-ir. PDH is known to shift the phase of the locomotor activity at the late subjective day, when injected into the vicinity of the AMe in *L. maderae* (Petri and Stengl 1997, J Neurosci 17:4097-4093). We wanted to know whether PDH as well as the colocalized FMRFAmid-like peptide phase delays the circadian pacemaker via suppression of action potential activity in pacemaker cells.

In extracellular recordings of isolated AMae we tested *in vitro* whether AMe-neurons generate circadian rhythms in their action potential frequency and whether PDH- and FMRFAmide peptides affect their electrical activity. We excised AMae with associated PDH-ir neurons with sharp glass pipettes and recorded from the explants with suction electrodes (0,3-2 MOMEGA). Recordings were performed under constant darkness, peptide injections were performed at specific Zeitgeber times with respect to the light dark cycles of the animal cultures. Tetrodotoxin (TTX)-blockable electrical activity was recorded for up to 5 days. We usually obtained multiunit recordings with several ampli-

tudes ranging from 30 - 90 muV, but occasionally we also obtained single unit recordings of 110 muV. The maxima of event-frequency occured at different Zeitgeber times for different units, but often preceded light-on or light-off. The activity oscillated with periods ranging from 6 h - 23.3 h. Thus, AMe neurons show oscillating electrical activity with different periods and varying phase relationships.

To test whether PDH- and FMRFAmide-like peptides affect the electrical activity of circadian pacemaker candidates we applied 150 fM of peptides with a micro injector to the excised AMe. In preliminary recordings we observed rises as well as decreases in electrical activity after application of PDH to different AMe cells at the late day. So far, we only observed increases in electrical activity after application of 100 fM FMRFAmide at the middle of the day. We currently test whether peptide actions are restricted to specific Zeitgeber times and to specific physiological types of AMe neurons and whether the peptide effects can be simulated by cyclic nucleotides. [Supported by DFG grants STE531/7-3 and Human Science Frontiers]

Peptide Interplay and Rodent Sleep 779

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It was hypothesized recently that the compex use of neuropeptides is much more natural and effective than their single administration because the peptide continuum of the entire body is the base for the functional control (Koroleva, Ashmarin. J.Theor.Biol. Vol. 216, No. 3, 257-271, 2002). Theoretical search using mathematical vector analysis revealed several peptide combinations capable to exert a pronounced psychotropic (anxiolytic) effect, including a combination of the δ sleep-inducing nonapeptide (DSIP) with a more prolong neuropeptide-tyrosine (NPY), consisting of 36 amino-acid residues. As antiphobic effects usually combine with stress-protective and sleep-promoting ones, we firstly studied the above-mentioned paired combination of DSIP and NPY administrated directly to the lateral ventricle of rabbits preliminary implanted (under local procain anesthesia) with intracranial electrodes for EEG of the cerebral cortex, hippocampus and olfactory bulb and EMG of the neck muscles as well as a single intracerebroventricular cannula. Otherwise the same animals treated with a saline were used as controls. Peptides dissolved in a saline were injected through the cannula at a volume of 60-80 ul just before the 12-hr dark period in experimental chambers. Paperless polygraphic recording started immediately since administration and lasted for 24 hr. Smaller dose of NPY (0.7 nmol) induced a slight increase in slow wave sleep (SWS) percentage during the first half of subjective night as compared to the controls (+25 min; $p<.05$; U-test), followed by its significant decrease during the second half (-53 min; $p<.05$), so clear decrease in SWS was seen for the entire dark period (-28 min; NS). Higher dose of NPY (7 nmol) induced higher SWS increase during the initial part of the dark period (+29 min; $p<.05$) followed by lower decrease, so total effect on sleep for the entire 12-hr dark period was zero. Finally, combined administration of NPY (7 nmol) and DSIP

(30 nmol) induced significant increase in SWS percentage for the initial 6 recording hrs (+57 min; $p<.05$) though later decrease smoothed; so the balance for the entire 12-hr recording period was clearly in favor of the increase in SWS (+53.5 min; $0.5<p<0.1$). Administration of DSIP alone revealed (in accordance to our earlier data, see: Kovalzon V.M. In: Y.Shimonishi, ed. Peptide Science - Present and Future. Dordrecht: Kluwer, 1999, p.757-758) only minor changes in sleep (+21 min for 12 hrs; NS). It is known that both DSIP and NPY can suppress the stress hormonal axis (see: Steiger A., Holsboer F. Sleep, 1997, v.20, N11, p.1038-1052; Antonijevic I.A., Murck H., Bohlhalter S. et al. Neuropharmacology, 2000, v.39, p.1474-1481). So the results confirm the initial hypothesis and, probably, reflect secondary participation of the administrated peptides in sleep-wake control which follow their involvement into endocrine processes.

780 Effects of Pituitary Adenylate Cyclase Activating Polypeptide (PACAP) in a rat model of diffuse axonal injury.

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PACAP is a member of the VIP/secretin/glucagon peptide family and expressed throughout the central and peripheral nervous system. Since its discovery, evidence has proved its neurotrophic and neuroprotective potential. *In vitro*, PACAP stimulates the growth and survival of neurons, prevents apoptosis and protects neurons against cytotoxocity induced by various agents (1, 2). PACAP has been reported to prevent ischaemic death of hippocampal neurons in rat global ischaemia (3) and decreased the infarct size after transient middle cerebral artery occlusion (MCAO)(4, 5). Similar results were achieved with PACAP in different models of traumatic brain injury (TBI). mRNA of endogenous PACAP was found elevated after experimental TBI using a rat contusion model (6). On the other hand, neither PACAP nor PAC1-R receptor mRNA was upregulated in a cortical stab injury model (7). The aim of the present study was to investigate the neuroprotective potential of PACAP in a rat model of diffuse axonal injury. The dose of PACAP administered to male WR rats weighing 350-400 g was calculated upon our previous observations in a rat model of MCA occlusion (4). In the present study, each rat was treated with a single bolus of 125 microg per bodyweight or vehicle via penis vein 5 minutes before injury. Immunohistochemistry with anti-APP as primary antibody (marker of altered axoplasmatic transport) was used to assess axonal damage. Immunopositive axon profiles in the area of corticospinal tract (CSpT) and medial longitudinal fascicle (FLM) were counted using the NIH Image J software. Student-t test was used to compare the density of immunoreactive profiles between PACAP-treated and control animals. Our results indicate that 2 h post-injury the density of APP immunoreactive axon profiles was lower after PACAP pretreatment both in the CSpT and in the FLM but the difference was not significant. However, 6 h post-injury the density of immunoreac-

tiv axonal segments appeared higher in the PACAP-treated group both in the CSpT and FLM. The difference was not significant neither in CSpT nor in FLM. The results of the present study should be considered rather surprising, particularly in light of the above-described beneficial effects of PACAP detected in ischaemic brain injury and cerebral contusion. Upon these observations it should be stressed that those therapeutic interventions proved to be efficient in brain ischaemia and contusion are not necessarily useful in the treatment of DAI. Alternativle, PACAP may only delay the axonal alterations after injury and may play an important role in the prolongation of the therapeutic window. Further investigations should resolve the controversy whether the intravenous administration protocol or the dosage applied in our present study are responsible for the results observed or, alternatively, PACAP is not effective in the pathobiological processes associated with/operant in DAI.

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4,5-Diaminofluoroscein Imaging of Nitric Oxide Synthesis in Crayfish Terminal Ganglia 781

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Local circuits in the central nervous system are subject to continuous modulation that matches their outputs to the specific requirements of an ever-changing environment. The terminal abdominal ganglion of the crayfish coordinates tailfan movements in locomotion and escape responses. Many studies have shown that the free radical nitric oxide (NO) plays a major role in circuit modulation. NO is synthesised from L-arginine, nicotinamide adenine dinucleotide phosphate and oxygen by the enzyme nitric oxide synthase (NOS). It then diffuses readily from its point of origin to act on its main target, the enzyme soluble guanylyl cyclase.

In the crayfish NO upregulates synaptic transmission to specific ascending intersegmental interneurons that innervate water-motion-sensitive hairs on the tailfan, while down-regulating or depressing synaptic inputs to another class of intersegmental interneurons (Aonuma and Newland, J Exp Biol 204:1319-13, 2001). NADPH diaphorase histochemistry is commonly used as an indirect method to reveal the presence of NOS activity in the central and peripheral nervous systems, but in invertebrates its specificity and use has recently been questioned. We have investigated NO synthesis in the terminal abdominal ganglion using the fluorescent probe 4,5-Diaminofluoroscein diacetate, DAF-2 DA. Following DAF-2 loading, reproducible cell-specific patterns of fluo-

rescence appeared in the ganglia, with strongly fluorescent cell bodies in specific anterior, central and posterior regions. We found that pre-incubation with the nitric oxide synthase inhibitor L-NAME reduced DAF-2 fluorescence levels in the ganglia, whereas the inactive isomer D-NAME had no effect. Washout of pre-incubated L-NAME caused increased cell-specific fluorescence due to endogenous NOS activity. Application of the NOS substrate L-arginine also resulted in an increase of DAF-2 fluorescence. Bath application of the NO donor SNAP resulted in DAF-2 fluorescence increases in most cells. We therefore presume that the cell-specific pattern of DAF-2 fluorescence indicates the distribution of neurones actively synthesising NO.

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782 Chronobiological quantification of pigment-dispersing factor in the cockroach *Leucophaea maderae*

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Circadian rhythms in behavior or physiology can be found in almost all organisms. Circadian rhythmicity is regulated by a circadian pacemaker that can be entrained by environmental cues. Pacemaker neuron releases output factors to signal circadian time to the rest of the body. Anatomical and lesion studies have suggested that neurons immunoreactive for the neuropeptide pigment-dispersion factor (PDF) are identical to the circadian pacemaker neurons in the brain of several insects [1]. In *Drosophila*, PDF acts as an output factor of the pacemaker neurons [2,3]. In *Leucophaea maderae*, PDF is postulated to be involved in the output and in coupling of the bilateral pacemaker neurons located in the accessory medulla [4,5]. The authentic PDF of *L. maderae* has not yet been characterized.

We thus isolate a PDF-like substance from an extract of 1000 *L. maderae* brains. The extract was separated into minute fractions by RP-HPLC. Fractions were tested for immunoreactivity by ELISA (using β–PDH antibody) and assayed for pigment-dispersing activity in destalked fiddler crabs. The fraction with highest immoreactivity also contained the highest bioactivity. This fraction was further purified by three separation steps and monitored by ELISA. The resulting PDF-like immunoreactive material has a mass identical to the PDF of *Periplaneta americana* and *Acheta domesticus* as determined by MALDI-TOF mass spectrometry.

In the pacemaker neurons of *Drosophila*, PDF immunoreactivity shows circadian fluctuation in the terminals in the dorsal protocerebrum [6], the likely circadian output area of the brain. In order to find the output area of the PDF neurons in *L. maderae*, we measured immunostaining intensity in three areas in the brain, plus in the lamina and medulla at different time points. In PDF cells of the anterior and the posterior medulla, staining intensity varied between different time points. The variation, however did not

seem to be circadian. We could not detect significant fluctuation of PDF staining in the lamina and the PDF neuron terminals in the central brain.

Currently, we are continuing our efforts to quantify the PDF-like immunoreactive material from HPLC purified extracts of brains and optic lobes at different time points by ELISA.

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PDF-containing clock neurons in the brain of *Drosophila* larvae express functional acetylcholine receptors

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Molecular components of the biological clock are already present during the embryonic development of *Drosophila melanogaster*. Expression of the clock genes *period* and *timeless* cycles in a circadian fashion in the larval CNS [1]. Although larvae obviously lack behavioural rhythms [2], it was found that locomotory or eclosion rhythms of adult flies raised under constant darkness could be synchronized by light pulses given during any of the larval stages [3-5]. These findings showed that i) larvae possess a time-memory (Zeitgedächtnis) which persists until the adult stage, and ii) light can entrain the circadian clock already in first instar larvae. Two types of photoreceptors are involved in larval light entrainment: the blue-light sensitive protein cryptochrome located within in the clock neurons [6], and the larval Bolwig organ (BO) [5]. The BO connects to the brain via the Bolwig nerve (BN) which makes synaptic contacts with the lateral neurons (LNs) [1,7]. The LNs constitute the master clock in the fly brain and express the neuropeptide pigment-dispersing factor (PDF). Axons of the BN express choline acetyltransferase [8], indicating biosynthesis of acetylcholine (ACh). Thus, ACh is a major candidate for signal transmission between the BO and the LNs, or, in other terms, a likely input factor of the *Drosophila* clock subserving light entrainment.

To test this hypothesis, we used Ca^{2+} imaging in short-term cultures of dissociated larval CNS neurons. The GAL4/UAS-technique allowed the identification of LNs among the neurons obtained through dissociation of whole larval brains by specific expression of green fluorescent protein. ACh induced an increase of the free intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ in the LNs which was dependent on Ca^{2+} influx. Nicotine and pilocarpine mimicked this ACh-induced increase of $[Ca^{2+}]_i$. Dissociated LNs could be immunostained with antibodies against different nicotinic ACh receptors subunits. Confocal imaging of whole-mounted larval brains revealed prominent immunostaining against these different nAChR subunits at the site of the synaptic contacts between the LNs and the BN. Our results provide pharmacological and immunonological evidence for a role of ACh as a circadian input factor in larval *Drosophila*. The ACh-induced increase of $[Ca^{2+}]_i$ in the LNs might be involved in the circadian regulation of clock gene expression or PDF release.

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784 The effect of serotonin on the Ih current of deep cerebellar nucleus neurons

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Serotonergic (5-HT) fibers originating from the brain stem project to the cerebellar cortex, the deep cerebellar nuclei, the inferior olive, and the pons. This indicates that the serotonergic system can modulate the signal processing performed by the cerebellar system. Evidence for the functional relevance of such a modulation comes from clinical studies that described improved motor performance of ataxic patients after application of 5-HT precursor substances. We were particularly interested in the effect of 5-HT on neurons of the deep cerebellar nuclei (DCN) because of the central position that the DCN have as the major output structure of the cerebellum.

We performed whole cell patch clamp recordings in para-saggital slices of the rat cerebellum. In current clamp recordings the input resistance of DCN was increased by 5-HT (10 µm) at hyperpolarized membrane potentials (n=7) pointing towards a blockage of the hyperpolarization activated Ih current by 5-HT. In voltage clamp experiments (EPC8) synaptic inputs, Na^+, K^+, and Ca++ currents were blocked pharmacologically and the command potential was stepped in 5 mV increments from ?40 mV to potentials between ?45 mV and ?100 mV. The Ih current started to be activated at ? 50 mV and reached its half maximal activation at ?70 mV. These values are without correction for a possible liquid junction potential that might result from a K-Gluconate based intracellular solution. Testing 10 µm and 20 µm of 5-HT we found a concentration dependent reduction of the Ih current by 5-HT (n=4). The Ih current was reduced to 75% of its control value by 10 µm 5-HT (n=9).

The role of the Ih current in DCN neurons is currently unknown, but it might potentially influence the generation of rhythmic activity patterns like in thalamic relay cells or it might effect the integration of synaptic inputs as suggested for cortical pyramidal cells. Effects of 5-HT on the Ih current are known from several neuron types, while a reduction rather then an increase of the Ih current by 5-HT has been reported only for Purkinje cells (Li et al., 1993, Brain Research 617:87). For Purkinje cells it has been suggested that the Ih current weakens the effect of inhibitory inputs. A reduction of the Ih by 5-HT in turn might increase the impact of GABA inputs on Purkinje cell hyperpolarization. Such a function could be of considerable relevance for DCN neurons considering

the dominant inhibitory projection that DCN neurons receive from Purkinje cells of the cerebellar cortex. Further studies will address the role of the Ih current and its modulation by 5-HT for the signal processing of DCN neurons.

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Brain plasticity, biogenic amines and aggression in the ponerine ant *Harpegnathos saltator* 785

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Aggression is a widespread phenomenon in both vertebrates and invertebrates. Despite its omnipresence, the neuronal basis of aggressive behavior is yet hardly understood.

In the present study, we began to investigate neuronal correlates of intraspecific aggression in the ponerine ant *Harpegnathos saltator*. In contrast to most other ant species, workers of *H. saltator* can mate and, therefore, produce queens and workers besides males. (Peeters et al. 2000, Insectes Sociaux 47:325-332) Thus, the life-time of a colony can be prolonged after the death of its queen by these inseminated egg-laying workers (gamergates). To acquire the status of a reproductive individual (i.e. of a gamergate), workers display highly aggressive interactions. These interactions are induced by chemosensory cues following the loss of either the queen or gamergates and are restricted to a particular time window.

The fact that intraspecific fights can be reliably triggered by the removal of reproductive individuals offers a great advantage to study neuronal correlates of aggression. Previous work showed that the transition from a worker to a gamergate is accompanied by an overall reduction in brain neuropile volume indicating long-term changes in the central nervous system (Gronenberg and Liebig, 1999, Naturwissenschaften 86:343-345). The switch in the behavioral display from a non-aggressive to a highly aggressive worker, however, occurs after only a few hours. This suggests that the changes in behavior are triggered by factors acting in a short-term range. Neuromodulators such as biogenic amines are known to modify the status of neuronal networks and are able to bias motor outputs, among other stages of neuronal processing. Most evidence in invertebrates comes from studies on Crustaceans indicating an association of biogenic amines and aggression (Kravitz, 2000, J Comp Physiol 186:221-238) We, therefore, started to investigate the neuronal representation of the biogenic amines serotonin, dopamine and octopamine in the course of the establishment of new reproductive individuals in the ant, *H. saltator*. An essential first step in our investigations was to scrutinize the innervation pattern of non-aggressive workers using immunofluorescent staining and laser-scanning confocal microscopy. The results constitute the basis for subsequent comparisons with aggressive workers and established gamergates. Biogenic-amine-containing neuronal processes were found in essential neuropile structures such as the central complex, the antennal lobes and the mushroom bodies. In contrast to previous findings in the honeybee and locust, serotonergic neurons exhibit a prominent innervation of the olfactory

input region of the mushroom body calyces. This suggests a potential role of serotonin in affecting olfactory information processing, and hence influences on a sensory system that is known to affect aggressive behavior to a great extent.

786 Splice variants of the cation channel LTRPC2 (TRPM2) are differentially activated by ADP-ribose and hydrogen peroxide

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The mammalian homologues of the *Drosophila* photoreceptor transient receptor potential (TRP) channel consist of a large and diverse family of proteins, which are found in a variety of organisms, tissues, and cell types, including nonexcitable and neuronal cells. It has been proposed that TRP channels participate in capacitative calcium entry (CCE). This is a a major pathway for Ca^{2+} entry into the cell, in addition to voltage-gated Ca^{2+} influx.

We identified four splice variants of the long transient receptor potential channel 2 (LTRPC2; also known as TRPM2), a member of the distantly related TRP channel proteins, which is expressed mainly in the brain. To analyze the function of the splice variants of LTRPC2, we expressed them heterologously in HEK 293 cells and studied their activation by ADP-ribose and NAD^+ with the patch clamp technique. A Stimulation of LTRPC2 by ADP-ribose and NAD^+ has been reported recently. Only when the full-length form was expressed, a stimulation by ADP-ribose was observed. However, the finding of an activation by NAD^+ was not confirmed.

Since ADP-ribose can be formed from NAD^+ and NAD^+ is elevated during oxidative stress, we tested the hypothesis that the various LTRPC2 channels mediate cation influx in response to an oxidant. As a model of oxidative stress, we therefore measured the currents after stimulating the cells with hydrogen peroxide (H_2O_2).

A cation influx partly carried by Ca^{2+} was induced by H_2O_2 in cells transfected with full-length LTRPC2 or transfected with one of the four splice variant, which had a deletion in the C-terminus, LTRPC2-Δ-C. No such current was observed in control cells nor in cells transfected with one of the variants which had deletions in other regions of the protein. Measurments of the free intracellulary Ca^{2+} concentration ($[Ca^{2+}]_i$) by the fluorescent dye fura2 showed a rapid increase of $[Ca^{2+}]_i$ after stimulation with H_2O_2 in LTRPC2-transfected cells as well as in control cells. Only in LTRPC2- or in LTRPC2-Δ-C transfected cells, H_2O_2 evoked a second delayed rise in $[Ca^{2+}]_i$. We conclude that the protein domain absent in LTRPC2-Δ-C is not necessary for its function as a Ca^{2+}-channel. The stimulation of LTRPC2-Δ-C exclusively by H_2O_2 provides evidence that H_2O_2 and ADP-ribose act by distinct mechanisms. Furthermore, intracellular neuroprotective signaling mechanisms after oxidative stress, induced by Ca^{2+}, may be mediated by the activation of LTRPC2 channels in response to an oxidant.

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Activation of metabotropic receptors elevates mitochondrial Ca^{2+} and stimulates oxidative metabolism in rat hippocampal slice cultures: Functional implications of cellular Ca^{2+} entry and release 787

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Metabotropic receptors modulate numerous cellular processes by intracellular Ca^{2+} signaling, but less is known about their role in regulating mitochondrial metabolic function within the CNS. In this study, we demonstrate in area CA3 of organotypic hippocampal slice cultures that glutamatergic, serotonergic, and muscarinic metabotropic receptor ligands, namely t-ADA, α-Me-5-HT, and carbachol, transiently increase mitochondrial Ca^{2+} concentration ($[Ca^{2+}]m$) as recorded by changes in Rhod-2 fluorescence, stimulate mitochondrial oxidative metabolism as revealed by elevations in NAD(P)H fluorescence, and induce K^+ outward currents as monitored by rapid increases in extracellular K^+ concentration ($[K^+]o$). Carbachol (1 - 1000 µm) elevated NAD(P)H fluorescence by up to 14 % F/F0 and increased $[K^+]o$ by up to 4.3 mM in a dose-dependent manner. Carbachol-induced responses persisted in Ca^{2+}-free solution and blockade of glutamatergic and nicotinic receptors by CNQX/D-AP5/mecamylamine. Under similar conditions (Ca^{2+}-free/CNQX/D-AP5) caffeine, known to cause Ca^{2+}-induced Ca^{2+} release (CICR), also evoked elevations in $[Ca^{2+}]m$, NAD(P)H fluorescence and $[K^+]o$ that, in contrast to carbachol-induced responses, displayed oscillations. Following depletion of intracellular Ca^{2+} stores by carbachol in Ca^{2+}-free solution, re-application of 1.6 mM Ca^{2+}-containing solution triggered marked elevations in $[Ca^{2+}]m$, NAD(P)H fluorescence and $[K^+]o$. These data indicate that metabotropic transmission effectively regulates mitochondrial oxidative metabolism via diverse receptor types in hippocampal cells, and that either i) InsP3-mediated Ca^{2+} release (IICR), or ii) CICR, or iii) capacitative Ca^{2+} entry (CCE) might suffice in stimulating oxidative metabolism by elevating $[Ca^{2+}]m$. Thus, activation of metabotropic receptors might significantly contribute to generation of ATP within neurons and glial cells.

Physiologically based Hodgkin-Huxley model simulates spiking behaviour of honeybee Kenyon cells 788

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The mushroom body within the insect brain is important for olfactory processing and associative learning. Kenyon cells are the intrinsic neurons of the mushroom bodies, and

thus, understanding the properties of Kenyon cells is an important step in understanding information processing and learning. To investigate the properties of Kenyon cells, we performed voltage- and current-clamp recordings from Kenyon cells *in vitro* and we developed a mathematical model of the Kenyon cell based on Hodgkin–Huxley equations. The parameters for the model were extracted from published data (Pelz et al., 1999 J. Neurophysiol. 81:1749; Grünewald 2003, J. exp. Biol., 206:117) and from our recent voltage- and current-clamp experiments. Using the patch-clamp technique in the whole-cell configuration we were able to switch between current-clamp and voltage-clamp measurements from individual cells, and thereby determine both their spiking behavior and analyze the underlying ionic currents.

Kenyon cells were not spontaneously spiking. Spikes could be elicited by current injection in ~90% of all recorded cells. Longer current injections (1 s) resulted in repetitive spiking in about 60% of the cells, while other cells remained silent after one initial spike. Our model incorporated all known voltage-dependent membrane conductances and accurately simulated the measured voltage- and current-clamp data. Kenyon cells express a fast transient Na^+ current, a delayed rectifier K^+ current, a *shaker*-like, transient K^+ current, and Ca^{2+} currents. From these experimental data, we determined the steady-state parameters (half maximal activation and inactivation) and the time constants of activation, inactivation, and recovery from inactivation. We also implemented a leak conductance based on measurements of the passive membrane properties of the Kenyon cells. The model was implemented and tested in the software package 'Simulator for Neuronal Networks and Action Potentials', version 7 (SNNAP 7.0, available at http://snnap.uth.tmc.edu/). It generated a membrane resting potential of -75mV and spikes as well as spike trains upon current injections. We investigated the role of the shaker-like K^+ current in determining spike shape and repetitive firing. The shaker-like potassium current had a predominant role in regulating spike duration, which supports the experimental finding that 4-AP block induces spike broadening.

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789 Characterization of the distribution patterns of HCN isoforms in rodent nasal epithelium and construction of targeting vectors for HCN1 and HCN4 knock out mice

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I_h currents are generated by hyperpolarization activated cyclic nucleotide gated (HCN) channels. These proteins are coded by a single gene in invertebrates, and by four (HCN1-4) in vertebrates. Activated by both hyperpolarization and cyclic nucleotides, they show a weak selectivity for K^+ over Na^+. I_h is crucial in setting the resting membrane potential and in the control of cell excitability, and is present ubiquitously in many locis of the heart and brain. With relevance to chemical senses, I_h channel mRNA has been detected in the antenna of various arthropods, in the olfactory bulb (HCN1,2,4), as well as in taste cells of rat vallate papilla (HCN1,4). Also, native I_h currents were characterized in the ORNs of rat, lobster and honey bee. It has been suggested that the effects of I_h on odor sensitivity and on the output of ORNs of rat in response to an odo-

rant is modulated by dopamine. In order to investigate the relevance of I_h channels for odorant transduction in vertebrates, we plan to make knock out mice for HCN1 and HCN4. For the purposes of a conditional gene targeting event, we are constructing a targeting vector such that exon 2 (HCN1) and exon 4 (HCN4) are flanked by loxP sites. This mouse line will then, after homologous recombination, be crossed with a lineage expressing the recombinase (Cre), resulting in the deletion of the target gene segment only in offspring cells expressing Cre. Further, to identify the different distribution of the four mammalian HCN isoforms in the olfactory system, slices from rodent nasal epithelium as well as single ORNs were investigated.

Characterization of novel homo- and heterooligomeric ligand gated chloride channels in *D. Melanogaster* 790

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Ligand gated ion channels (LGIC) mediate the fast responses of neuronal and muscle cells to neurotransmitters. They form a large family of cation channels that can be activated by acetylcholine (ACh), serotonin and anion channels that are opened by γ--amino-butyric- acid (GABA) and glycine. In addition glutamate, histamine and serotonin gated anion channels exist that can only be found in invertebrates. An universal feature of this type of LGIC subunits is a common topology of four membrane spanning segments (M1--M4) and a huge N--terminal extracellular domain with an hyperconservated cystein sequence motive. The M2 transmembrane segment, that forms the pore in the functional pentameric channel complex, determines the ion selectivity (unspecific cation or chloride). Recently there was considerable progress in the characterization of ligand-gated chloride channels in invertebrates due to the cloning of histamine and serotonin gated channels from *Drosophila* and *C. elegans*, but

the molecular basis of several other types of ion channels known from electrophysiological studies, like ACh- and multitransmitter-gated anion channels is completely unknown.

The wealth of accumulating information from the *D. melanogaster* genome sequencing project has given us the possibility to describe all members of the superfamily of ligand gated ion channels used by an individual species. A systematic analysis reveals that 10 genes are supposed to code for nACh receptors or similar ligand gated cation channels and 12 genes code for GABA, histamine, glutamate receptors or related ligand gated chloride channels predicted on the base of homology to already identified channels. Our main interests are the systematic investigation of the members of the chloride channel group for which the ligand is still unknown and the elucidation of the possible subunit composition of the ion channels.

Using reverse transcription-polymerase chain reaction the transcripts of several putative ligand gated ion channels predicted from genome sequence data were cloned. In order to

investigate the function of the cloned cDNAs, RNA or expression plasmids were microinjected singly ore in combination into Xenopus oocytes. Oocytes were recorded in the two-electrode voltage clamp configuration and tested for responses to several agonists. By systematically testing single cDNAs as well as combinations, application of different stimuly evoked currents in Oocytes showed the functional expression of a completely novel type of ligand gated ion channel. Some other types of ligand-gated ion channels respond to multiple neurotransmitters and co-expression of ion channel subunits leads to hetromultimeric channels with altered properties.

791 Characterization of I_h channels from invertebrate olfactory receptor neurons

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Cyclic nucleotides play an essential role in visual and olfactory transduction in many species. We therefore used RT-PCR to discern the presence of ion channels that are gated or modulated by cyclic nucleotides in lobster, honeybee and fruit fly olfactory receptor neurons (ORNs). ORNs of all three species express homologs of members of the I_h (HCN, HAC) family of ion channels that are activated by both hyperpolarization and cyclic nucleotides. When recombinantly expressed in HEK293 cells, cloned AMIH (*A. mellifera*), DMIH (*D. melanogaster*) and PAIH (*P. argus*) gave slowly activating, non-inactivating inward currents under whole-cell voltage-clamp in response to hyperpolarizing voltage steps. cAMP shifted the activation curve to more positive values within the normal resting potential range. The general properties of all three Ih channels were similar, but the channels differed in critical details. In particular, cAMP shifted the activation curve of PAIH significantly more than AMIH. Native I_h-currents in lobster and honeybee ORNs mimicked the recombinant ones. Western blotting localized the expression of PAIH to the olfactory sensilla. The physiological properties of these channels, and the presence of at least PAIH in the transduction zone, suggest a potential a role for these channels in olfactory transduction.

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Functional consequences of ε AChR subunit truncating mutations linked to congenital myasthenic syndrome

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Congenital myasthenic syndromes (CMS) are inherited disorders due to presynaptic, synaptic, or postsynaptic defects of neuromuscular transmission. Some previously described kinships with typical signs of CMS showed a marked deficiency of nicotinic acetylcholine receptors (nAChR) as well as biochemical and morphological changes at the neuromuscular junctions. Recently two truncating mutations in the muscular nAChR e-subunit gene (*CHRNE*) were identified in two affected families (Sieb et al. 2000). These mutations in the ε-subunit , ε911delT and ε1030delC, result in nAChR deficiency and affected end-plate maturation most likely as a result of loss in the fourth transmembrane domain of the receptor protein. To date, the functional consequences of the mutations are unknown. We introduced these two deletion mutations into the human ε-subunit and investigated their functional consequences after expression in human embryonic kidney 293 cells. Using whole-cell patch-clamp recordings we show that nAChR containing the ε911delT or ε1030delC mutation form functional channels, however, exhibit lower current density compared to wt-nAChR. Initial biophysical studies indicate alterations in desensitization properties of ε1030delC subunit containing nAChR. It is possible that these changes may underlie the defects in neuromuscular transmission observed in CMS.

Sieb et. al., Hum Genet 107:160-64, 2000

Nifedipine inhibits the delayed rectifier K^+ current in rat hippocampal and human neocortical neurons

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The pharmacological treatment of epilepsies is still not satisfactory. About 30 % of patients continue to have seizures despite appropriate medical therapy. Current antiepileptic drugs affect neuronal Na^+- or Cl^- -channels or interfere with glutamate or GABA metabolism. As epileptic discharge is also driven by Ca^{2+} influx into neurons, Ca^{2+}-channel blockers have been investigated for treatment options. Before suppressing epileptic activity in neocortical and hippocampal slices, application of nifedipine led to a transient increase in the repetition rate which might be due to blockage of K^+- channels (Straub et al., Neuroscience 2000; 95(1):63-72). Inhibition of the delayed rectifier potassium outward current (Ik,dr) by nifedipine has been shown for molluscan and embryonic murine neurons so far.

The aim of the present investigation was to identify the impact of nifedipine on the Ik,dr in mammalian adult neurons. Human cortical neurons obtained from brain surgery (epi-

lepsy or tumor) and rat hippocampal neurons of the CA1 region were dissociated and assessed using the whole-cell patch clamp technique. Na^+- currents were blocked by prior activation with a depolarizing prepulse from -80 mV to -50 mV; inhibition of Ca^{2+}-currents occurred by application of 2 mM $CoCl_2$. Whole-cell K^+- currents were evoked by a depolarizing step from -50 mV to 0 mV. In the presence of nifedipine Ik,dr was reversibly blocked in a dose-dependent manner. Dose-response curves revealed an IC_{50} of 420 nM (rat) and 80 nM (human), respectively. 20 mM TEA, a known K^+- channel blocker, was as effective on blocking the Ik,dr as 0.3 µm nifedipine in both rat and human neurons.

Thus, in the concentration range tested (0.3 µm to 100 µm) nifedipine inhibits Ik,dr in human and adult rat neurons and therefore provides an explanation for the transient increase in repetition rate of epileptic discharges mentioned above.

794 Expression of NCKX but not NCX correlates with the kinetics of glutamate responses and expression of AMPA receptors in rat histaminergic neurons

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The different roles of Na^+/Ca^{2+} (NCX)- and Na^+/Ca^{2+} /K^+ (NCKX)- exchangers in the regulation of ionic homeostasis in neurons is poorly understood. Little is known about stoichiometry, localization and function of different subtypes of these exchangers. With the help of nested single-cell RT-PCR (scRT-PCR) we determined coexpression patterns of different subunits of NCX and NCKX with AMPA receptors and serotonin receptors in rat histaminergic tuberomamillary (TM) neurons. We found, that NCX1, NCX2, NCKX2 and NCKX3 are expressed in TM neurons. NCKX2 occurred only in TM cells with fast desensitizing glutamate responses, where it was coexpressed with the GluRD subunit in its flop splice variant. NCKX3 was detected only in neurons lacking the GluRD-flop splice variant. NCX expression did not correlate with the GluR subunit expression. AMPA receptors in TM neurons were found to be Ca-impermeable. Serotonin-receptor expression did not correlate with the expression of NCX and NCKX. Immunostaining with NCKX2 antiserum revealed dense dendritic or/and axonal localization of this protein in the TM region. We conclude that the expression of NCKX2 and NCKX3 correlates with the AMPA receptor- type, which may reflect either a coupling between AMPA-receptors and the NCKX exchanger or different mechanisms for maintaining calcium homeostasis in the neurons with fast or slow glutamate responses.

Effect of hyposmotic conditions on cell volume and electrophysiological properties of leech Retzius neurones 795

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Changes in cell volume can disturb the normal function of animal cells. Therefore most cells are endowed with volume-regulating mechanisms. A decrease in the extracellular osmolyte concentration leads to hyposmotic conditions and thus to cell swelling. Under these conditions many cell types tend to counteract swelling by an activation of volume-sensitive ion channels, permitting a loss of osmolytes.

We investigated the effect of hyposmotic conditions on cell volume of leech Retzius neurones using ion-selective microelectrodes, and on the electrophysiological properties using the current-clamp and the voltage-clamp technique. We found that a decrease in the extracellular osmolarity of 40% (achieved by omitting the appropriate amount of NaCl from the bathing solution) induced a cell swelling by 40 ± 3% (n = 4), a hyperpolarisation by -5.5 ± 6.5mV (n = 15), and a decrease in the input resistance by 37 ± 20% (n = 11). Voltage-clamp experiments revealed that the decrease in input resistance was most prominent near the resting potential. To verify that these effects were not caused by the reduction of NaCl or ionic strength, Na^+ and Cl^- were replaced by non-permeable ions (NMDG and gluconate) or by saccharose. Under these isosmotic conditions, the hyperpolarisation was similar to that in hyposmotic conditions, while cell volume and input resistance remained almost unchanged. These results suggest that the decrease in input resistance is caused by an activation of volume-sensitive ion channels.

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Pressure Injection: A Reliable Method to Determine Cytosolic Buffering in Single Cells? 796

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The applicability of pressure injection to study the intracellular ionic buffering capacity was investigated by injecting electrolyte or buffer solutions from glass capillaries into Retzius neurones of the leech *Hirudo medicinalis* or oocytes of the frog *Xenopus laevis*. The resulting changes in the intracellular free ion concentration ($[Na^+]_i$, $[Cl^-]_i$, $[Mg^{2+}]_i$) were measured simultaneously with ion-selective microelectrodes.

A major difficulty was the exact determination of the injected volume. Initially it was attempted to inject the respective solutions into oil and to determine the diameters of the resulting droplets. Control experiments showed, however, that this method was inaccurate for glass capillaries with tip diameters < 0.5 µm. Detailed calculations support the hypothesis that the error can be explained by the surface tension between electrolyte droplet and oil, which retards droplet formation at the tips of the glass capillaries. These effects increase with decreasing tip diameters.

To avoid these problems, in further experiments either the very large frog oocytes were used, as they tolerated impalement with comparatively blunt glass capillaries (tip diameter > 1 µm), or the volume ejected from the glass capillaries was estimated by injection into an electrolyte droplet of known volume and a simultaneous determination of the resulting changes in ion concentrations with ion-selective microelectrodes.

For Na^+ and Cl^- it could thus be demonstrated that their removal from the cytoplasm of leech Retzius neurones was so efficient that only a small fraction of the injected ions actually contributed towards an increase in the cytosolic concentration. Previous studies had already shown that Mg^{2+} is strongly buffered in the cytosol of leech Retzius neurones. When injected into frog oocytes, $MgCl_2$ and EDTA yielded similar values for intracellular Mg^{2+} buffering. These values appear to reflect true cytosolic buffering, as they were not affected by the disruption of the mitochondrial membrane potential and only slightly affected by the inhibition of the Na^+-dependent Mg^{2+} extrusion.

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797 Permeation of Ca^{2+}, Sr^{2+}, and Ba^{2+} through the caffeine-sensitive cation channels in leech P neurons

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Leech P neurons possess caffeine-sensitive cation channels in their plasma membrane and in intracellular organelles (Schoppe et al. 1997). Activation of the channels causes an increase in the cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$), which is transient, most probably due to the desensitization of the channel-activating mechanism. The desensitization is virtually irreversible, as indicated by the ineffectiveness of subsequent caffeine applications. To characterize the Ca^{2+} influx through the caffeine-sensitive cation channels in more detail we determined the dependence of the caffeine-induced $[Ca^{2+}]_i$ increase, measured by Fura-2, on the extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$). At low $[Ca^{2+}]_o$, up to 30 µm, the caffeine-induced $[Ca^{2+}]_i$ increase was exclusively due to intracellular Ca^{2+} release. The increase was small, ranging between 50 and 150 nM, reached its peak after 25-50 s, and recovered by 50% within 50-200 s. At higher $[Ca^{2+}]_o$ the caffeine-induced $[Ca^{2+}]_i$ increase became progressively larger due to Ca^{2+} influx. The Ca^{2+} influx seemed to be maximal at $[Ca^{2+}]_o$ = 300 µm, since at this $[Ca^{2+}]_o$ the caffeine-induced $[Ca^{2+}]_i$ increase had reached a maximal value of about 1 µm. $[Ca^{2+}]_i$ reached its peak after 15-25 s and recovered by 50% within 30-50 s. Increasing $[Ca^{2+}]_o$ further up to 57 mM had no significant effect on amplitude or time course of the caffeine-induced

$[Ca^{2+}]_i$ increase, indicating that within this concentration range channel desensitization and active Ca^{2+} extrusion are independent on $[Ca^{2+}]_o$.

Besides to Ca^{2+} the caffeine-sensitive channels are permeable to various other divalent cations such as Co^{2+}, Ni^{2+}, Mn^{2+}, Zn^{2+}, Cd^{2+}, and Mg^{2+} (Schoppe et al. 1997), and, as expected, we found them to be also permeable to Sr^{2+} and Ba^{2+}. In the presence of extracellular Sr^{2+} the time course of the caffeine-induced changes in the Fura-2 fluorescence was very similar to that recorded in Ca^{2+}-containing solutions, and after a single exposure to caffeine subsequent caffeine applications were ineffective. These results show that in the presence of Sr^{2+} channel desensitization is unaffected, and that Sr^{2+} is actively extruded from the cytosol with a similar speed as Ca^{2+}. Contrastingly, in the presence of Ba^{2+}, the caffeine-induced changes in the Fura-2 fluorescence were clearly different from those in Ca^{2+}-containing solutions. In particular, recovery of the fluorescence changes and hence active Ba^{2+} extrusion was very slow. Furthermore, a second caffeine application evoked a distinct Fura-2 response, albeit with reduced amplitude, indicating incomplete desensitization of the channel-activating mechanism.

Reference:
Schoppe, Hochstrate & Schlue (1997), Cell Calcium 22:385-397

Properties of Ca^{2+}-activated large conductance K^+ channels in mammalian inner hair cells 798

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Auditory inner hair cells (IHCs) transduce cochlear vibrations into receptor potentials that trigger synaptic activity of the IHC and finally evoke postsynaptic action potentials in afferent auditory neurons. The IHC's voltage response is determined by K^+ conductances, which are carried by Ca^{2+}- and voltage-dependent large conductance K^+ channels (BK channels) and purely voltage-dependent channels (K_v- and KCNQ-type channels) (1,2). In particular, the BK current is thought to be pivotal for shaping the receptor potential due to its fast activation kinetics and large size (3).

We examined the properties of pharmacologically isolated BK channels in IHCs from acutely isolated mouse and rat cochleae under whole-cell voltage-clamp conditions as well as in inside-out patches at defined intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$). In the whole-cell configuration, BK currents displayed a steep voltage dependence of 8 mV for an e-fold change in activation. Voltage at half-maximal activation (V_h) was -44.8 mV, in close agreement with data from guinea pig IHCs (1).

In excised inside-out patches, large BK currents of around 0.5 nA were obtained. When intracellular solutions with varying free $[Ca^{2+}]_i$ were applied to the cytoplasmic face of the patch, V_h shifted to hyperpolarised potentials with increasing $[Ca^{2+}]_i$ (V_h(0µM)= 39mV; V_h(1µM)= 7mV; V_h(10µM)= -69mV). Similarly, activation rates were accelerated with increased $[Ca^{2+}]_i$. Comparison with properties of BK channels from other tissues (5) indicates that the IHC variant of BK is characterised by a particularly negative activation range. The slope of voltage dependence (18 mV) was independent of $[Ca^{2+}]_i$ and considerably less steep than in whole-cell recordings.

Comparing the BK activation properties in whole-cell configuration with the patch data obtained at defined intracellular $[Ca^{2+}]_i$ allowed for an estimation of the intracellular Ca^{2+} concentration sensed by the K^+ channels in the intact IHC. Firstly, the increased slope under whole-cell conditions indicated a progressive increase in $[Ca^{2+}]$ with depolarization that is likely to result from activation of the IHC Ca_V channels. In perfect agreement with the very negative activation of BK channels, the Ca_V channels of IHCs are formed by $\alpha 1_D$ (Ca_V 1.3) that characteristically opens at potentials as low as -65 mV (4). Secondly, high (µMolar) $[Ca^{2+}]_i$ was reached already at relatively negative potentials. Thus, estimated Ca^{2+} was about 2 µm at -65 mV and exceeded 10 µm at -20 mV. Such high $[Ca^{2+}]_i$ concentrations in the vicinity of the BK channels might be indicative of a close colocalisation with Ca^{2+} channels.

(1) Kros & Crawford (1990) J Physiol 421:263;
(2) Oliver et al. (2003) J Neurosci in press;
(3) Kros et al. (1998) Nature 394:281;
(4) Platzer et al. (2000) Cell 102:89
(5) Cui et al. (1997) J Gen Physiol 109:647

799 Possible role of TRPC channels in nociceptive processing

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Sensory neurons express a variety of Gq/11-coupled receptors which upon stimulation activate the diacyl glycerol/protein kinase C (DAG/PKC) signalling pathway. In addition, many of the mediators that activate this cascade lead to an increase in intracellular Ca^{2+} concentration which up to now has been attributed to calcium release from internal stores. One alternative mechanism mediating this increase may be Ca^{2+} influx via members of the transient receptor potential (TRPC) ion channel family. Therefore, the presence of TRPC channels in rat sensory neurons was investigated at the transcriptional (RT-PCR), translational (immunohistochemistry) and functional (Ca^{2+}-imaging) level.

Primary cultures of sensory neurons were cultured in the presence of NGF and, after 1 day, were loaded non-disruptively with the calicum indicator dye FURA-2/AM. In microfluorimetric measurements, nociceptive neurons responded to the nociceptor stimulant capsaicin with a pronounced calcium influx. About one third of capsaicin sensitive neurons were also sensitive to stimulation with the membrane permeant DAG analog OAG (oleyl acyl glycerol). OAG induced calcium rises were abolished in the absence of extracellular free Ca^{2+} suggesting calcium influx as the predominant source of the calcium response. Furthermore, addition of the TRPC channel blockers Ni2+/Cd2+ (200 µm) or SKF 96365 (10 µm) or the protein kinase inhibitor staurosporin (1 µm) entirely prevented the calcium responses to OAG but not to capsaicin. Analysis of the total RNA of lumbar DRG revealed mRNA expression of al least five out of seven known TRPC channels (TRPC1, TRPC3-6). Indirect immunocytochemistry identified immunoreactivity for TRPC1-, TRPC3- and TRPC6-proteins in sensory neurons. TRPC3/TRPC6 have been found to be activated by OAG in previous studies. All TRPC

positive neurons were also immunoreactive for the nociceptor specific TRPV1 (vanilloid receptor).

Together our results suggest functional expression of TRPC1, 3 and 6 ion channels in rat sensory neurons. These ion channels may functionally couple to Gq/11-coupled receptors and finally activate calcium influx into nociceptive neurons. Both DAG production and PKC activation seem to be essential for this novel pathway of calcium signalling following Gq/11 receptor activation in nociceptive neurons. (DFG Projekt HA 3140/1; SFB 353, Teilprojekt A10)

Subtypes of nicotinic acetylcholine receptors modulate the intracellular calcium level in nociceptive neurons of the rat. 800

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Stimulation of nicotinic acetylcholine receptors (nAChR) on peripheral nociceptive neurons is followed by analgesia. The signalling pathways responsible for this effect are unknown. We investigated the localisation of nAChR a-subunits in nitric oxide (NO) synthesizing nociceptive DRG neurons and the changes in $[Ca^{2+}]i$ in response to nAChR-stimulation. Nociceptive, vanilloid receptor-1 (VR1)-immunoreactive, neurons showed also strong immunoreactivity for the nAChR α-subunits $\alpha3$, $\alpha5$ and $\alpha7$ and for the endothelial isoform of the NO-generating enzyme NO-synthase (NOS). Sensory neurons also coexpressed VR1, the $\alpha7$-subunit and the neuronal NOS. Isolated lumbar capsaicin-sensitive DRG neurons were stimulated with the nAChR agonists nicotine and epibatidine. In 40 % of these neurons, activation by nicotine or epibatidine was followed by an increase in $[Ca^{2+}]i$. The blocker of nAChR, mecamylamine, abolished the effect in all of these neurons whereas the blocker of $\alpha7$-nAChR homopentamers, methyllycaconitine inhibited the effect in about 50 % of the nicotine-sensitive nociceptive cells. Blockade of voltage-gated Ca^{2+}-channels by verapamil and inhibition of a4b2-nAChR by dihydro-ß-erythroidine inhibited the nicotine/epibatidine-induced increase in $[Ca^{2+}]i$ only in 5 % of these nociceptive neurons. The results suggest the presence and involvement of multiple nAChR subtypes ($\alpha3\beta4$, $\alpha7$) in the increase of $[Ca^{2+}]I$ of individual nociceptive neurons. This could stimulate one or both NOS isoforms with subsequent NO synthesis and the reduction of excitability (DFG, HA3140/1,2).

801 Localisation of the endogenous toxin-like modulator lynx1, and its relation to the nicotinic acetylcholine receptor subunit α7 and α10 in rat ganglia

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Lynx1 is a neuronal membrane molecule related to snake α-neurotoxins. In brain it colocalises with α4β2 and α7 nicotinic acetylcholine receptors (nAChR). Electrophysiological studies indicated that lynx1 is able to promote the larger of three current amplitudes elicited by acetylcholine and that it enhances desensitization and increases the EC_{50} for ACh in α4β2 nAChRs. On this background, we investigated the occurence and distribution of lynx1 and nAChR subunits α7 and α10 in rat dorsal root ganglia (DRG) and ganglia of the sympathetic system with RT-PCR and immunofluorescence. By using gene-specific primers for lynx1, RT-PCR products of the appropriate size were amplified from rat DRG, superior cervical ganglion, stellate ganglion, celiac and superior mesenteric ganglion and lumbar sympathetic ganglia. In immunofluorescence analyses a granular and predominantly intracellular labelling pattern was observed in all neuronal perikarya with a monoclonal antibody against lynx1. Double-labelling immunofluorescence demonstrated colocalisation of lynx1 with nAChR subunits α7 and α10-immunoreactivities. These data show a widespread distribution of lynx1 in the peripheral nervous system, both in neurons that receive synaptic input from cholinergic terminal (sympathetic neurons) and in neurons carrying exclusively extrasynaptic nAChRs (DRG-neurons).

802 Voltage-Dependent Potassium Channel in Rat Sensory Neurones is Blocked by Hypoxia

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The lower oxygen level is during ischaemia of the peripheral nervous system one of the suggested mechanism underlying the observed phenomenon of paraesthesia and pain. Oxygen is, beside its essential nature for survival of neurones, known for various mechanisms activated on the cellular level, which includes in other organs ion channels.

We used the enzyme-free thin-slice preparation of dorsal root ganglia (DRG) as model to measure small to medium sized neurones of rats (2-9 d) by means of the patch-clamp technique. The solutions were aerated with different oxygen concentrations which were measured in the perfusion system with a Paratrend-7 system.

In the whole-cell configuration we measured in 65% of all neurones a reduction of about 32% of the mean outward current amplitude (at +60 mV) in Ringer solution by moderate hypoxia (10-28 torr) within 3-5 min. The blockade of voltage-dependent K^+-currents amplitude was prevented in external cholin-Cl solutions in presence of 50 nM Dendrotoxin (DTX). To elucidate the channel subtype we used other toxins as Dendrotoxin-K, Margatoxin and Titiustoxin-K-α, but none of the substances could abolish the reduction. TEA at 5 mM could not prevent a further K^+ current reduction in hypoxia.

In about 60% of the neurones we found a increased action potential duration and in few neurones a reduced firing frequency. Therefore we conclude that the rapid blockade of voltage-dependent K^+ currents by moderate hypoxia which were Dendrotoxin sensitive might contribute to clinically observed phenomenons. We suggest from the toxin data that the underlying K^+ current is a heteromer of Kv genes.

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The characterisation of 5-HT7 receptor isoform: Specific receptor-G-protein interaction and post-translational modifications

803

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The 5-HT7 receptor is the most recently identified member of the family of G-protein coupled serotonin receptors. Functionally, this receptor is involved in circadian rhythm, sensory processing and regulation of limbic processes. The 5-HT7 receptor is also known to have a high affinity for a several antidepressants. A possible involvement of 5-HT7 receptor in migraine and in the modulation of sympathetic afferent pathways further increase the interest for this receptor subtype.

The aim of this scientific project was to investigate the expression and function of the 5HT7a receptor isoform in two different modell systems, i.e. insect cell (Sf9) and mammalian cells (COS 7 and N1E). We also analysed receptor interaction with G-proteins and downstream signalling cascades. As palmitoylation represents common feature of G-protein coupled receptor, it was under the interest to determine whether 5-HT7 is palmitoylated and to evaluate functional activity of this modification.

We found that 5-HT7 is posttranslationaly modified with palmitate in agonist-dependent manner. We also defined the acylation sites by site-directed mutagenesis. To analyse the function of palmitoylation all mutants were analysed for the cAMP accumulation and for the efficiency of G-protein coupling. Results obtained from these experiments indicate that palmitoyltion is critically involved in receptor function and in particularly in regulation of basal activity.

804 Functional role of acylation of 5-HT$_{1A}$ receptor.

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The mouse 5-HT$_{1A}$ receptor was expressed with a baculovirus system in insect cells and analysed for acylation. [^{3}H]palmitic acid was efficiently incorporated into 5-HT$_{1A}$ and the label was sensitive to a treatment with reducing and nucleofilic reagents indicating a thioester-type bond. Surprising, acylation was inhibited by cycloxehimide, indicating that only newly synthesised 5-HT$_{1A}$ can be acylated. Treatment of the infected cells with 5-HT didn't reveal any dose-dependent change in the [^{3}H]palmitate incorporation. Taken together, our data demonstrate that the acylation of 5-HT$_{1A}$ is rather irreversible process and that stimulation with agonist does not change the turnover rate for the receptor-bound palmitate.

Two conservative cystein residues 417 and 420 in the carboxyl terminus of 5-HT$_{1A}$ were identified as the acylation sites by site-directed mutagenesis. Both of them are palmitoylated at virtually same extent. To adress functional role of 5-HT$_{1A}$ acylation we analysed an ability of acylation-deficient mutants to interact with G_i. Both the basal binding and the binding upon stimulation were significantly reduced after replacement of one or both of the acylation sites. It suggests that the acylation of 5-HT$_{1A}$ plays functional role in the receptor signalling.

805 Post-translational modifications and functions of 5-HT4 receptor.

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Serotonin (5-hydroxytryptamine or 5-HT) is an important neurotransmitter involved in a wide range of central and peripheral physiological functions. A number of different G-protein coupled 5-HT receptors are known to sensitively modify different neuronal networks by their specific action on synaptic transmission and postsynaptic excitability. Here we demonstrate that the mouse 5-HT4(a) receptor is modified by covalently attached palmitic acid. We found that (i) palmitoylation is a reversible process and (ii) agonist stimulation of the 5-HT4(a) receptor increases the turnover rate for receptor-bound palmitate. Analysis of acylation-deficient mutants revealed the ability of palmitoylation to modulate the agonist-independent, constitutive 5-HT4(a) receptor activity through Gs pathway. In addition, we showed for the first time that 5-HT4(a) receptor is coupled not only to Gs, but also to heterotrimeric G13 protein. Our data suggest that dynamic palmitoylation of the 5-HT4(a) receptor could play a role of molecular switch differentially regulating Gs- as well as G13-mediated constitutive receptor activities by dictating conformation of receptor's flexible cytoplasmic loops. We also propose that by

activating 5-HT4(a) receptor/G13 pathway, serotonin may play a prominent role in regulating neuronal architecture in addition to its classical role in neurotransmission.

In addition, we shown for the first time that the 5-HT4(a) receptor undergoes posphorylation. Experimental data suggest the importance of GRK kinases in this process, whereas PKA and PKC seems to be not involved. The level of phosphorylation was increased upon agonist stimulation in a dose-depended manner. Interestingly that phosphorylation efficiency was tightly correlated with the extent of receptor palmitoylation: removing of acylation sites resulted in increased basal as well as agonist-mediated phosphorylation. Functionally, interplay between these two post-translational modifications may be critically involved in the receptor desensitization.

The interaction of κ M-conotoxin RIIIK with *Shaker* potassium channels from trout

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Cone snails are predatory marine animals which capture their prey by injecting highly efficient venom into the victim. The venoms usually consist of up to 200 different usually cysteine rich peptides – the conotoxins. Conotoxins are highly specific, extremely potent and relatively easy to be synthesized. Due to these features they are important probes for the investigation of structural and functional properties of the target molecules which are mainly ligand gated and voltage gated ion channels. Two decades ago, mu -conotoxin GIIIA was the first conotoxin shown to target voltage-gated Na^+-channels from skeletal muscle. Later, κ -conotoxin PVIIA, was the first conotoxin identified to specifically block voltage-gated K^+ channels. The cysteine pattern of the mu -conotoxins and κ -conotoxins is different and accordingly these peptides belong to different superfamilies of conopeptides. Recently, a new conotoxin was isolated from *C. radiatus*, which has a disulfide pattern similar to the mu -conotoxins, but has an entirely different pharmacological specificity: Instead of interacting with Na^+ channels it blocks voltage gated K^+ channels. Therefore, this conotoxin is the first member of new conotoxin family, the κ M-conotoxins.

In this project, the interaction of κ M-conotoxin RIIIK on the *Shaker*-related K^+ channel from trout (*Tsha1*) was investigated. *Tsha1* K^+ channel are blocked by κ M-conotoxin RIIIK in a state-dependent manner with an IC_{50} for the closed state of about 20 nM. To identify the key residues of the κ M-conotoxin RIIIK important for the binding to *Tsha1* channels an alanine scanning mutagenesis was performed. Except for the cysteines residues all the amino acid residues of the toxin were individually mutated into an alanine. By using the *Xenopus* oocyte expression system, we estimated the affinities of the different κ M-conotoxin RIIIK mutants to the *Tsha1* K^+ channel by means of the 2-electrode voltage clamp technique. Although most of the the alanine mutations had only

a minor effect on the affinity, we were able to identify 4 residues where the alanine mutants had a more than 20-fold reduced affinity to *Tsha1* K^+ channel. These results define the interaction surface of the peptide toxin with the ion channel protein and indicate that the interaction seems to be quite complex since several amino acids of the peptide are important for the interaction. Furthermore this study may provide also some insights into potential structural links between the conotoxin members of the mu -, κ - and κ M-conotoxin families.

807 Expression of heteromeric Kv1 potassium channels in *Xenopus* oocytes

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It is well known that voltage activated Kv 1 potassium channel members have the ability to form heteromeric channels that consist of up to four different α subunits. This heteromerization provides one mechanism underlying the great variety of K-channels with different properties observed. It has been shown that heteromerization is a common process in normal tissue, but also occurs in expression systems like the *Xenopus* oocyte system. In order to estimate the amount of different heteromultimers expressed upon coinjection of RNA encoding different Kv1 α subunits a model has been proposed that assumes that the formation of the channels depends only on the relative amount of the different RNA injected (Hopkins W.F. 1998, J. Pharmacol. Exp. Ther., 285). If this model is true for the injection of two different RNAs the number of the different possible homomeric and heteromeric channels expressed should follow a binominal distribution. Based on that coinjection of RNA encoding a fast inactivating (Kv1.4) and a non inactivating (Kv1.1) channel subunit should lead to a current which shows an increasing inactivation time constant and an increasing steady state current with an increasing relative amount of RNA encoding Kv1.1. Since the inactivation of Kv1.4 mediated currents is complete the steady state current is a direct measure for the amount of homomeric non-inactivating Kv1.1 channels.

In the study presented here we try to answer the question whether the formation of heteromeric Kv1 family channel members follows a simple stochastic model in the *Xenopus* expression system or if more complex ways of channel formation are present. In order to achieve this goal different mixtures of RNA encoding Kv1.1 and Kv1.4 were co-injected into *Xenopus* oocytes and the resulting currents were recorded by using the two-electrode voltage clamp technique. The ratios of the amount of RNA encoding Kv1.1 to the amount of RNA encoding Kv 1.4 were 1:2, 1:1, 2:1 and 4:1. The resulting currents were compared with the predictions made from the model assuming a binomial distribution. Based on the predictions of the model we expected that an increase in the relative amount of the RNA encoding Kv1.1 should lead to a slower inactivation time constant of the current and to an increase of the amount ot the steady state current. The data obtained show that the amount of the steady state current measured behaved as predicted by the model. In contrast to this the coinjection of the two RNA led to a slowing in the inactivation time constant which did not vary with the relative amount of Kv1.1. In summary our data provide some evidence that the formation of Kv1.1/Kv1.4

heteromeric channels expressed in *Xenopus* oocytes is stochastic. On the other hand our data do not rule out the possibility of other more complex mechanism involved within the expression of heteromultimeric channel.

Immunocytochemical localization of $P2X_3$ receptor subunits in the rat brain 808

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P2X receptors are ligand-gated ion channels expressed in a variety of excitable cells including peripheral and central neurons. Extracellular ATP acting through P2X receptors mediates fast synaptic transmission and presynaptic modulation of neurotransmitter release. Seven members of the P2X receptor family have been cloned ($P2X_1$-$P2X_7$) of which mainly $P2X_2$, $P2X_4$ and $P2X_6$ subunits have been detected in rat brain. These findings do not satisfactorily explain the abundance and wide distribution of alphabeta-meATP binding sites in the rat brain and the detection of alphabeta-meATP elicited currents since only $P2X_1$ and $P2X_3$ containing receptors are sensitive to this agonist. So far, $P2X_3$ immunoreactivity has only been detected in the NTS, the solitary tract and the spinal trigeminal nucleus (1). To determine the distribution of $P2X_3$ receptor subunits in the rat brain, antibodies were raised in rabbits against its C-terminal domain. The subunit specificity of the affinity-purified $P2X_3$ antibody was tested on Western blots of crude membranes isolated from cells transfected with all P2X receptor subunits. Only in HEK-293 transfected with $P2X_3$ cDNA, a triplet band of 60.8, 57.9 and 54.6 kDa was detected. Western blots of crude brain membranes of P5-P26 rats showed a unique band with an apparent molecular weight of 64.4 kDa.

Immunohistochemistry was carried out on coronal and sagittal sections of rat brain (P21). Controls were performed either by omission of the first antibody or by preincubating the first antibody with the corresponding fusion protein. In both cases no immunoreactivity was observed confirming the specificity of the assay. In cerebellar cortex, Purkinje cells showed strong immunoreactivity , with labeling extending from the cell body to the dendrites. Weak staining was observed in the granular cell layer. In the hippocampus, pyramidal and non-pyramidal bodies and dendrites in the CA1 and CA3 region were stained. The strongest labeling was observed in the CA3 region while the dentate gyrus showed no staining. Strong immunoreactivity was observed in the cortex where mainly pyramidal neurons in layers II-VI were labeled. Weak to moderate staining was found in several regions of the brain stem including the pontine, facial, mesencephalic motor, dorsal cochlear nuclei and the inferior olive. In the olfactory bulb, the strongest labeling was observed in the mitral cell layer, where mitral cells presented immunoreaction in both cell bodies and dendrites. In summary, the distribution of $P2X_3$ receptor subunits in rat brain determined by immunocytochemistry is more extended than previously thought. This suggests that $P2X_3$ subunits participate in the P2X receptor mediated response to ATP in the CNS.

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809 Persistent Upregulation of Thalamic Alpha-2B Adrenoceptors After Chronic Psychosocial Stress

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Alpha-2 adrenoceptors are regulated by stress and have been hypothesized to be involved in the etiology of depressive disorders. Little is known about the Alpha-2 adrenoceptor subtype B which is strongly expressed in the thalamus. Also, few studies have addressed the question if stress-induced changes in the brain persist after the end of a stressful period. Using the stress paradigm in male tree shrews, an animal model for depression, we monitored thalamic Alpha-2 adrenoceptors both after a six-week period of chronic psychosocial stress and after 10-days *post* stress. [^{3}H]RX821002 receptor autoradiography and *in situ* hybridization with an Alpha-2B adrenoceptor probe were performed on frontal brain sections to assess changes in protein and gene expression. In stressed animals, body weight decreased and urinary noradrenaline and cortisol levels were increased during the stress period but normalized *post* stress. In the brain, chronic psychosocial stress (1) leads to an increase in Alpha-2 adrenoceptor binding in thalamic nuclei (2) with elevated Alpha-2B adrenoceptor mRNA levels in the paraventricular nucleus. Furthermore, (3) the stress-induced alterations exacerbate post stress. The upregulation of Alpha-2B adrenoceptors indicates low levels of noradrenaline both during and after the prolonged stress period. Together, these changes might influence neurotransmission between thalamus and limbic brain areas, contributing to the etiology of depressive disorders.

810 Block of AMPA-type glutamate receptor channels by the novel antagonists RPR119990 and RPR 117824

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Antagonizing glutamatergic neurotransmission by the blockade of AMPA type glutamate receptors is a promising pharmacological strategy for neuroprotection in neurodegenerative diseases like Amyotrophic Lateral Sclerosis (ALS) or Parkinson`s disease (PD). We investigated the interaction of two new pyrazine derivatives (RPR 119990 and RPR 117824) with native and recombinant AMPA type glutamate receptors. Recombinant human homooligomeric GluR1 flop, GluR2 flip, GluR2 flop, GluR6, non-desensitizing L506Y GluR2 channels and heterooligomeric GluR1/2 channels were expressed in HEK293 cells. In addition, we used neuronal autaptic cultures to investigate the impact of the two new substances on synaptic transmission.

Coapplication of the drugs with 10 mM glutamate resulted in a slight dose-dependent depression of the peak current amplitude by 10-20 % at a concentration of 1 mM. The current decay in presence of glutamate was faster in presence of the blockers. Resensitization of GluR1 or GluR2 receptor channels follows a monoexponential time course with a time constant in the range between 10 ms and 150 ms. Resensitization had a biexponential course when glutamate and the blocker were coapplied. The slower component of recovery was independent on blocker concentration and had values of around 2 to 4 s, at least two orders of magnitude slower than the fast resensitization. The proportion of the second, slower component of recovery on the whole recovery process increased with blocker concentrations and reached 90% at GluR 2 flip, and 50% at GluR1 flop and GluR2 flop channels at 1 mM. The non-desensitizing L506Y GluR2 channels showed a marked, dose-dependent current decay upon coapplication of glutamate and RPR119990/RPR117824. A similar slow component of recovery was found as compared to wild-type GluR2 channels in the presence of the blockers.

At kainate-type GluR6 receptors no block was observed upon coapplication of glutamate and RPR119990/RPR117824 in single and double pulse experiments.

AMPA-type channels were competitively blocked by RPR119990 or RPR117824 with an IC50 around 10nM, at GluR6 channels the dose-response relation of the observed competitive block was shifted to higher concentrations. Similar IC50 values for synaptic transmission could be obtained in cultured neurons upon application of the respective antagonists during basal stiumulation conditions.

In conclusion, our results show a specific block of recombinant AMPA-type GluR channels and synaptic AMPA receptors by these novel pyrazine derivatives. There is evidence from the kinetic analysis of the block effect that more than one blocked states exist in AMPA-type receptors. We performed a quantitative analysis of the experimentally observed block mechanism by computer simulation and could predict the experimental results by the addition of blocked states connected with the unliganded closed state and liganded states of the receptor in a Markov model. Especially the IC50 in the low nanomolar range at AMPA-type glutamate receptors points to a potential role of the novel drugs in preventing glutamate-triggered excitotoxic cell damage and goes along with positive results from pharmacological testing at the transgenic model of familiar ALS.

Ligand-gated chloride channels of the fruitfly *Drosophila melanogaster* 811

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Inhibitory neurotransmission is essential for the proper function of all brains. In vertebrates, GABA and glycine are the substances that transmit inhibitory signals. In invertebrates, including insects and worms additional compounds such as glutamate, histamine and serotonin serve as inhibitory transmitters. To learn more about the molecular ratio-

nal underlying inhibitory neurotransmission in general, we characterized all putative ligand-gated chloride channels of the fruitfly *Drosophila melanogaster*. We identified a total of 12 genes in the genome of *Drosophila*. Expression analysis revealed that at least 11 of them are transcribed at detectable levels. Four of them, the GABA-receptors GRD, Lcch3 and Rdl as well as the Glutamate gated chloride channel were already well characterized. Recently, we were able to identify the two histamine-gated chloride receptor subunits, DmHACl-1 and DmHACl-2. All others (CT 19189, CT 21430, CT 23187, CT 23391, CT25610, CT 5896) are less well studied and their cognate ligands are not known. When comparing their sequence signatures, only the CT 25610 could be grouped into the GABA-receptor family, while the others share greater similaritis with the glutamate-gated chloride channels known from other organisms.

Homologues to vertebrate glycine receptors are not believed to be present in *Drosophila*, as their closest homologs are the two HA-gated chloride channels. To learn more about the role of these receptor subunits, we performed expression studies with all candidates, which revealed exiting differences in there expression patterns. Some of them show a very broad expression in neuronal as well as in non-neuronal tissues. Among them are e.g. the Rdl, the Grd, the CT 19189 and the CT 23187 subunits. Others, such as the Dm HACl-1 show a more interesting expression pattern, as these transcripts could exclusively be detected in cells that are postsynaptic to photoreceptors, including the lamina monopolar cells. The expression pattern of the second histamine-gated chloride channel remains so far enigmatic, as results derived from *in situ* hybridizations appeared somehow inconsistent and less expoundable. Another group of receptors is exclusively present in the nervous system without expression in the peripheral organs. Furthermore the last group shows a very attractive expression pattern as only neuronal subpopulations are labeled. Thus, the CT 21430 transcript shows almost excusive expression in the antennae and the optic lobes.

Currently, transgenic animals carrying constructs that enable RNAi-mediated gene silencing under the control of UAS-sequences, are under construction. They consist of inverted repeat structures carrying at least two introns to allow almost complete silencing. In addition to the so far accumulated data, these transgenic flies will certainly allow us to identify the physiological significance of these receptors in defined parts of the brain as well as in different peripheral organs and to shed light into the complex functions of these receptors.

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Analysis of tissue distribution, pharmacology and physiological significance of octopamine receptor splice variants of the fruit fly *Drosophila melanogaster*

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Octopamine (OA) and tyramine (TA), biogenic monoamines, are the invertebrate counterparts of adrenaline and noradrenaline. They act as neurohormones, neuromodulators and neurotransmitters in various invertebrates. TA is the biological precursor of OA and is present in relatively high concentrations in neuronal as well as in non-neuronal tissues of most invertebrate species studied. The known actions of OA in the central nervous system include desensitization of sensory inputs, influence on learning and memory, or regulation of the `mood´ of the animal. OA and tyramine are the only neuroactive non-peptide transmitters whose physiological role is restricted to invertebrates, which focused the interest of pharmacologists on the corresponding receptors. All known octopamine and tyramine receptors are members of the great GPCR receptor family.

Although numerous studies were performed with the aim to analyze the physiological role of OA in invertebrates, our information regarding octopaminergic neurotransmission in *Drosophila* is fragmentary especially taking into account the tremendous possibilities of this model organism. Mutants defective in the synthesis of OA are already available, but unfortunately, they show not the expected phenotypes. This might be due to plastic processes, where TA activates OA receptors thus concealing the expected phenotypes. To learn more about octopaminergic neurotransmission in *Drosophila* and the aminergic neurotransmission in general, we performed different types of experiments including *in situ* hybridization, heterologous expression and we are currently constructing fly lines that allow RNAi-mediated gene-silencing of the OA receptor gene. These flies carry an inverted repeat construct of the OA receptor gene spaced by two introns, whose transcription is under the control of UAS-elements. This allows, taking advantage of the Gal4/UAS-system, to silence the octopamine receptor in special tissues or parts of the nervous system.

Expression profiling of two out of the three different octopamine receptor splice variants revealed differential patterns. Whereas one of the splice variants is primarily transcribed in the peripheral tissues, the second one shows a ubiquitous transcription in neuronal and non-neuronal tissues. It appears that the majority of neurons in the brain express the corresponding receptor, which is in contrast to the situation found for the transmitter OA, which is present in only a small subset of cells. To evaluate if the different splice forms of the receptor have different physiological roles, they are currently expressed in heterologous systems. Using Xenopus oocyte expression as well as expression in human cells, the pharmacological differences between the splice variants as well as their intracellular coupling is studied.

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813 Physiological and molecular analysis of muscarinic neurotransmission in the nematode *Caenorhabditis elegans*

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Acetylcholine is a major neurotransmitter in the entire animal kingdom. It functions as the major transmitter at the neuro-muscular synapse, both in vertebrates and in some invertebrates such as nematodes. Beside the nicotinic acetylcholine receptors (nAChR) that are the major type of ACh receptor, muscarinic ACh receptors are known for their diverse modulatory actions. Regulation of nicotinic neurotransmission at the neuro-muscular synapse is a well known action of muscarinic ACh receptors. In the nematode *Caenorhabditis elegans*, a total set of three different muscarinic acetylcholine receptors was described. The aim of this study is to identify those receptors that transmit effects that could be attributed to muscarinic neurotransmission.

The effects of muscarinic ACh receptors could easily be quantified at the pharynx of the worm. The pharynx muscles show a stereotype contraction leading to pumping movements of the pharynx, which is required to ingest bacteria, the main food of these worms. Activation of the muscarinic system leads to an increased pumping rate, blokking of this system to a decreased pumping rate. To identify the receptor that is responsible for these actions, we performed gene-silencing experiments with all three candidate genes. Only one, the mach receptor III showed the expected phenotype. Silencing of both other genes had no effect on the pharyngeal pumping. In addition, muscarinic agonists (oxotremorine) and antagonists (atropine) had no effect on the type III receptor depleted worms. Silencing of both other receptors did not change the susceptibility for either oxotremorine or atropine. This clearly indicates that only the type III mACh receptor transmits the known effects of ACh via the muscarinic pathway. Regarding the susceptibility of the worms depleted from the other candidate genes against muscarinic agonists and antagonists, it is highly probable that they are no muscarinic ACh receptors. Phylogenetic analysis strongly support this hypothesis. In addition, the intracellular coupling of the single remaining muscarinic ACh receptor was studied using silencing of all relevant Ga-protein subunit genes. Only worms defective in the expression of the Gqa-protein subunit showed the expected phenotype, making it highly probable that the Gqa-protein acts downstream of the muscarinic ACh receptor. To follow the signal pathway a PLC mutant was examined regarding to pharyngeal pumping. These β-phospholipase c deficient worms show no response to oxotremorine and atropine. So you can summarize that the type III mACh receptor is coupled to the PLC-pathway. To get more information about the cellular expression of the type III mAChR in hermaphrodites, a transgenic line with GFP under the control of the receptor promotor was made. The interneurons I2, I3, I4 and I6 as soon as the motorneuron M2 express the type

III mAChR. These findings allow to describe the interaction of muscarinic neurons which synergistically evoke pharynx contractions.

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Kv3. 1- and Kv3. 4-mediated K currents in nerve terminals of the rat posterior pituitary 814

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The release of vasopressin and oxytocin from nerve terminals of the posterior pituitary is controlled by the frequency and shape of action potentials generated in two hypothalamic nuclei. Each of the neuropeptides is produced by one type of neuron, transferred by axoplasmic transport to the nerve terminals and secreted into the blood upon specific stimuli. Vasopressin and oxytocin cells respond to an increase in the osmolarity of the extracellular solution with a sustained increase in spike frequency. In addition, each cell type is able to exhibit a characteristic pattern of phasic firing.

One possibility to explain the differences in the firing properties of vasopressin and oxytocin neurons could be the existence of different sets of K^+ channels. In nerve terminals of the rat neurohypophysis three types of K current have been described so far, a delayed rectifying, an A-type transient and a Ca^{2+}-dependent K current (Thorn et al., J. Physiol. 432:313 (1991); Bielefeldt et al., J. Physiol. 458:41 (1992). We tried to identify some of the K channels involved at the molecular level and to assign them to one type of nerve terminal.

K channels were investigated in nerve terminals of the rat posterior pituitary using the patch clamp technique and immunocytochemistry. Two groups of nerve terminals could be distinguished. One group comprised about 70% of the nerve terminals and contained a delayed rectifying K current (IKD) and up to three types of transient K currents. IKD started to activate positive to -30 mV and exhibited half-maximal activation at -8 mV. The time course of activation could be described with voltage-dependent time constants. IKD is presumably mediated by channels composed of Kv3.1 subunits. The three transient K currents consisted of IKtf, a transient K current exhibiting fast and voltage-dependent inactivation, which was activated positive to -50 mV and half-activated at -28 mV. Another transient K current, IKts, activated at membrane potentials >0 mV and inactivated with slow voltage-independent kinetics (t~70 ms). The third transient K current was blocked by BDS I (IK-BDS), known to selectively block Kv3.4 channels. IK-BDS activated positive to -40 mV, was half-activated at -20 mV and exhibited voltage-dependent activation and inactivation kinetics. Kv3.1 as well as Kv3.4 channels were only present in nerve terminals containing vasopressin. In about 30% of the terminals the predominant K current was an A-type current (IKA), it activated positive to -60 mV, was half-activated at -7 mV and inactivated with voltage-independent kinetics (t ~20 ms). The molecular nature of the K channel mediating the A-type current is unknown.

815 Expression and function of erg K^+ channels in gonadotroph cells of the rat pituitary

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Transcripts of all three ether-a-go-go-related gene (erg) K^+ channels are expressed in the rat anterior pituitary (Wulfsen et al. 2000, J Neuroendocrinol. 12:263). With the help of single cell PCR, transcripts of all three erg channels have previously been detected in lactotroph cells which are known to exhibit erg currents involved in the TRH-induced secretion of prolactin (Schäfer et al. 1999, J Physiol. 518:401). We have now investigated the expression of erg1 in the different cell types of the rat anterior pituitary. Using primary cultures of dispersed rat pituitaries immunocytochemistry with antibodies against erg1 (Alomone Labs) resulted in a moderate staining of lactotroph cells and a strong staining of all gonadotroph cells as revealed by double staining with antibodies against prolactin or FSH and LH, respectively.

To perform electrophysiological recordings, gonadotroph cells were identified by their FSH secretion with the help of the reverse hemolytic plaque assay. Using the whole-cell patch-clamp technique typical erg currents were recorded in external solutions with high (140 mM) K^+ concentration in gonadotroph cells derived from male and female rats. The currents could be blocked completely and selectively by the methansulfonanilide E-4031. Using hyperpolarizing pulses from a holding potential of -20 mV, the inward-rectifying erg currents clearly dominated the recorded membrane currents. The voltage dependence of the time course of recovery from inactivation and deactivation of gonadotroph erg currents was similar to heterologously expressed rat erg1 currents. The voltage dependence of erg current activation was measured with 4 s depolarizing test pulses from a holding potential of -80 mV followed by a constant hyperpolarization to -100 mV. The maximal erg current amplitudes at the hyperpolarizing pulse were plotted as a function of the preceding test pulse potential. Isochronal activation curves were obtained by fitting the data with a Boltzmann function. The inflection potential indicating the halfmaximal activation of the erg currents was -30.6 ± 1.9 mV, $n = 8$. Using Ringer as external solution, the gonadotrophs exhibited a small sustained outward erg current in the range of the normal resting potential of non-oscillating gonadotrophs. In the current clamp mode, the application of E-4031 resulted in a clear depolarization of the membrane potential.

The combined immunocytochemical and electrophysiological results strongly suggest that gonadotroph cells express functional erg1 channels which are responsible for the maintenance of the normal resting potential of the cells.

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KCHIP interaction with a conserved retention signal containing n-terminal domain of Kv4 channels 816

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Kv channel interacting proteins (KChIPs) associate with Kv4 channels , which leads to a modulation of these A-type potassium channels. Our goal was to identify and characterize the KChIP interaction domain(s) on Kv4 -subunits. In patricular, we wanted to find out, if the Kv4/KChIP interaction is similar to the Kv1/Kvbeta interaction. The latter is well understood on a structural basis and involves a T1-loop structure of the -subunit.

By heterologous coexpression in mammalian cell lines and Xenopus oocytes we showed, using electrophysological and immunocytochemical methods, that KChIP2.2 modulates Kv4.2 inactivation gating and functional cell surface expression. KChIP2.2 slows the initial phase of inactivation and accelerates the recovery from inactivation. KChIP2.2 stimulates Kv4.2 current densities, which correlates with a redistribution of immunoreactivity from perinuclear areas to the plasma membrane and with higher amounts of Kv4.2 protein in the oocyte surface membrane. Increased Kv4.2 current densities were also obtained in the abscence of KChIP2.2, when the highly conserved proximal Kv4.2 N-terminus (40 amino acids) was deleted. The same domain is required for the association of KChIP2.2 with Kv4.2 -subunits, and therefore the N-terminal deletion mutant Kv4.2 40 shows none of the typically observed effects when coexpressed with KChIP2.2.

We hypothesize that the Kv4.2 proximal N-terminus bears a retention signal, which can be masked by the association of KChIPs. Functional expression data obtained with chimeric channels, in which the 40 amino acid domain was inserted at different locations within the channel protein, support this idea. Scanning mutagenesis within the 40 amino acid region will identify the amino acid residues involved in KChIP binding and ER-retention.

Loss of functional M-current-mediating KCNQ channels leads to abnormal excitability and resonance behaviour in hippocampus and neocortex 817

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Heteromeric voltage-gated potassium channel subunits of the KCNQ family are the molecular correlates of the native M-current that regulates subthreshold electrical excitability of many neurons. The M-current is thought to play a crucial role in the generation of medium afterhypolarisations (mAHPs) and early spike frequency adaptation in hippocampal pyramidal neurons.

Mutations in either KCNQ2 or KCNQ3 co-segregate with benign familial neonatal convulsions (BFNC), a neonatal form of epilepsy. Functional analysis of BFNC-associated mutations in heterologous expression systems revealed only subtle reductions in current amplitude. Disruption of the KCNQ2 gene in mice resulted in a lethal phenotype due to pulmonary dysfunction in homozygous knockout mice.

We have investigated the physiological role of the M-current by expressing a dominant-negative KCNQ2 subunit in mouse brain. The resulting suppression of M-channel activity in CA1 hippocampal pyramidal neurons reduced their early spike frequency adaptation and the corresponding afterhyperpolarizations (mAHPs) following action potential trains. The data also indicate that the characteristic θ frequency resonance behavior of the CA1 neurons is caused by M-channels operating in the subthreshold membrane potential range. The altered neuronal oscillatory properties and excitability were mirrored by abnormal rhythmic brain activity, learning deficits, network hyperexcitability, and by pronounced behavioral hyperactivity accompanied by occasional epileptic seizures. Our data provide new insight into the functional roles of M-channels in neuronal response patterns, cerebral network activity, and behavior.

818 Structural determinants of Kv4 channel inactivation

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Kv4 channels mediate both the cardiac transient outward current I_{to} and the neuronal somatodendritic A-type current. The special inactivation behaviour determines the functional role of Kv4 A-type channels. They can undergo transitions from the open state to an open-inactivated state, but also from closed states to closed-inactivated states. We used suitable mutant channel constructs to study Kv4 open-state and closed-state inactivation separately. Chimeric Kv2.1(4.2NT) channels, where the cytoplasmic Kv2.1 N-terminus had been replaced by homologous Kv4.2 domains, showed fast inactivation with typical features of the classical *Shaker* N-type inactivation. Kv4.2 channels, following fractional inactivation, mediated tail currents with biphasic decay, indicative of passage through the open state during recovery from inactivation. These results revealed typical N-Type inactivation features for Kv4.2 wild-type channels, whereas no such features were present in N-terminally truncated Kv4.2Δ40 channels. The closed-state inactivation was unaffected in N-terminal deletion mutants. We found that closed-state inactivation in the three members of the Kv4 subfamily had different kinetics, with Kv4.1Δ40 (τ = 790 ms at -50 mV) and Kv4.3Δ40 (τ = 1420 ms at -50 mV) beeing the extremes. Ongoing mutational experiments are being carried out to elucidate the structural basis of Kv4 closed-state inactivation.

Noncapacitative calcium current and calcium signaling in neurosecretory insect neurons

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Efferent dorsal unpaired median (DUM) neurons are neurosecretory cells containing the biogenic amine octopamine which is released into the periphery thereby modulating various structures such as skeletal and visceral muscles. The intracellular free Ca^{2+} concentration $[Ca^{2+}]i$ of DUM neurons from abdominal ganglia of the cockroach Periplaneta americana is regulated by a Ca^{2+} background current flowing through the membrane of the cell body. Octopamine and neuropeptides were found to modulate this current and thus cause a change in $[Ca^{2+}]i$.

Potentiation of the background Ca^{2+} current by the neuropeptide Neurohormone D (NHD), a member of the adipokinetic hormone family, enhances $[Ca^{2+}]i$ in ryanodine- and ruthenium red-sensitive manner suggesting an involvement of ryanodine receptors in the generation of these Ca^{2+} signals. We investigated the mechanism by which NHD enhances the background current using patch clamp and fluorescence microscopy. The potentiation of the background current by 10 nM NHD was strongly attenuated in the presence of the PLC inhibitor U37122 (3 μm). Block of IP3 receptors with heparin did not affect NHD action. However, when the DAG lipase was inhibited with RHC80267 (50 μm) NHD failed to enhance the background current. On the other hand, polyunsaturated fatty acids such as arachidonic acid (AA) and linoleic acid mimicked the effect of NHD. This indicates that NHD may act via production of AA from DAG. The electrophysiological results were confirmed by microfluometric measurements of $[Ca^{2+}]i$. NHD fails to elicit Ca^{2+} signals in the presence of U37122 and RHC80267, and AA dose-dependently enhances $[Ca^{2+}]i$. A role of PKG in the regulation of the NHD effect on background current could be excluded. The stimulating effect of NHD was not affected by the presence of the PKG inhibitor KT5823 (1 μm).

NHD did not potentiate the Ca^{2+} background current in the presence of LOE908 (40 μm), an inhibitor of noncapacitive Ca^{2+} currents. Thus the Ca^{2+} background current in DUM neurons might be related to noncapacitive Ca^{2+} currents. Noncapacitive Ca^{2+} channels are permeable to Ca^{2+}, Ba^{2+} and Sr^{2+}. This was also found for NHD which enhanced the membrane conductance in the presence of either Ca^{2+}, Ba^{2+} or Sr^{2+}. These findings give rise to the assumption that the rise background Ca^{2+} current in DUM neurons may represent a noncapacitive Ca^{2+} current.

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820 Ethosuximide and Mibefradil display differential blocking effects on low voltage-activated Ca^{2+} channels in thalamic neurons

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Low voltage-activated (LVA) or T-type Ca^{2+} channels are observed in many central and peripheral neurons and display distinct physiological and functional properties. During states of slow-wave sleep and absence epilepsy, thalamocortical relay neurons show rhythmic burst activity based on the activation of LVA Ca^{2+} channels. Since there is an ongoing discussion about the contribution of LVA channels to epileptic seizures and about the sensitivity of these channels to anticonvulsants like ethosuximide, we here compare the electrophysiological actions of ethosuximide and the highly selective T-type Ca^{2+} channel blocker mibefradil on LVA Ca^{2+} currents in identified thalamic cell types. Therefore we isolated two populations of neurons from the dorsal lateral geniculate nucleus (dLGN) of Wistar- and WAG/Rij-rats. The latter are considered as a genetic rat model of human generalized absence epilepsy. Whole-cell patch-clamp recordings on thalamocortical relay neurons and local GABAergic interneurons revealed a dose-dependent decrease of LVA Ca^{2+} current amplitude after application of both ethosuximide and mibefradil. Application of mibefradil resulted in a complete block of LVA Ca^{2+} currents with comparable IC_{50} values of ~1 µm for relay neurons and ~2 µm for local interneurons in both rat strains. Ethosuximide was proven to be less effective in blocking LVA Ca^{2+} currents. Application of ethosuximide resulted in a maximal reduction of about 40 % of current amplitudes with comparable IC_{20} values of ~1 mM for relay and interneurons in WAG/Rij rats. Preliminary data indicate higher IC_{20} values for neurons from Wistar rats. Since the three members of the LVA Ca^{2+} channel family ($Ca_V3.1$ - 3.3) differ in their electrophysiological and pharmacological properties, we investigated the expression of the three Ca_V3 isoforms by means of RT-PCR techniques. On tissue level we found a high expression of $Ca_V3.1$, a detectable expression of $Ca_V3.2$, and no expression of the $Ca_V3.3$ isoform in dLGN. Moreover in an *in vitro* model of intrathalamic oscillations, mibefradil was able to significantly dampen the strength of network activity.

Based on these data we would like to suggest that ethosuxmide and especially mibefradil are effective T-type Ca^{2+} channel blockers in thalamic neurons and have anti oscillatory and thus anti-epileptic potential. More generally we conclude, that the tissue distribution and the sub-cellular localization of LVA Ca^{2+} channel isoforms as well as their specific pharmacological sensitivity are essential parameters to understand the *in vitro* and *in vivo* effects of mibefradil and other anticonvulsants like ethosuximide. (Supported by Novartis-Stiftung and DFG - BU 1019/5-1)

Expression and electrophysiological properties of hyperpolarization-activated cation channels in a rat model of absence epilepsy

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Human childhood absence epilepsy (CAE) is a familial disease with a complex genotype. There is some evidence of a channelopathy with Ca^{2+} and K^+ channel anomalies being critically involved. The present study has been done to establish a possible role of Ih current mediated by hyperpolarization-activated cyclic-nucleotide-gated cation (HCN) channels in the epileptogenesis of WAG/Rij rats, a genetic model of CAE. Whole-cell patch-clamp recordings of thalamocortical relay neurons in brain slices, combined with RT-PCR as well as *in situ* hybridization, revealed the expression of all four HCN channel isoforms (HCN1-4) in the dorsal thalamus (including the lateral geniculate nucleus, dLGN, and the ventrobasal thalamic complex, VB) known to be a key region in the genesis of physiological and pathophysiological rhythmic activity in the thalamocortical system. As a control, all experiments were performed also in ACI rats, a rat strain known to be free of absence epilepsy seizures. In rats older than postnatal day 19, the half-maximal potential of Ih current activation (Vh) was significantly different in ACI (-88 mV) compared to WAG/Rij (-94 mV) rats. Application of cAMP resulted in a 5 and 2 mV positive shift in Vh in ACI and WAG/Rij rat, respectively. A similar shift could be obtained by application of the phosphodiesterase inhibitor IBMX. During current clamp recordings application of IBMX shifted the activity mode of thalamocortical relay neurons from burst to tonic firing only in ACI rat. These results point to a facilitated generation of rhythmic activity in the thalamocortical system of WAG/Rij rats. A semi-quantitative RT-PCR analysis on tissue level demonstrated an up-regulation of the HCN1 isoform in the dLGN region of WAG/Rij rats, although none of the other isoform expression had marked differences there. *In situ* hybridization analysis revealed a differential expression pattern of all HCN channel isoforms in the two rat strains. In summary, our data suggest that HCN channels are candidate genes that may contribute to epileptogenesis in WAG/Rij rats and, possibly, humans suffering from CAE. (Supported by DFG: BU 1019/5-1, Leibniz-Programm to HCP).

Ca^{2+}-dependent inactivation of neuronal Ca^{2+} channels: A restricting mechanism of an ubiquitous intracellular mediator

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One of the main mechanisms of Ca^{2+} entry into the cell involves the opening of Ca^{2+} channels in the plasma membrane. To effectively control Ca^{2+} signalling, Ca^{2+} channels inactivate rapidly by a mechanism that depends on an elevation of intracellular Ca^{2+}

within tens of nanometres of the channel pore. This mechanism to restrict the Ca^{2+} influx in neuronal cells, the Ca^{2+}-dependent inactivation of Ca^{2+} channels thus representing an autoinhibitory feedback. Recent physiological, biochemical and molecular studies have yieled new insights into the regulation of neuronal Ca^{2+} channels and especially into the Ca^{2+} dependent inactivation (CDI). Here we want to present a unifying model of this CDI process.

Focusing on an *in vitro* cell model we investigated the role of CDI in thalamocortical relay cells: Since large Ca^{2+} transients are present in thalamic neurons Ca^{2+} dependent mechanisms play a pivotal role for thalamic physiology. Studies combining electrophysiological and Ca^{2+} imaging techniques revealed strong increases in the intracellular Ca^{2+} concentration following high-voltage activated Ca^{2+} current activation. The expression of the $Ca_V1.2$ (L-type current), $Ca_V1.3$ (L-type), $Ca_V2.1$ (P/Q-type), $Ca_V2.2$ (N-type), and $Ca_V2.3$ (R-type) subunits could be demonstrated. Double-pulse experiments further revealed that Ca^{2+}-dependent inactivation in thalamocortical cells is mediated only by Q- and L-type Ca^{2+} currents. These channel subtypes are regulated in thalamic cells by the cAMP/PKA pathway, Ca^{2+} binding proteins, calmodulin and the cytoskeleton. Taken together our findings demonstrate, that different mechanisms regulating CDI are cooperative in a defined neuronal cell type.

823 Agonist-specific translocation and recycling of nucleotide-activated P2Y2 receptor.

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Extracellular nucleotide triphosphates especially ATP or UTP exert a large number of physiological responses mediated either by the inotropic P2X receptors or G-protein coupled P2Y receptors. Seven subtypes in this family namely P2Y(1,2,4,6,11,12,13) have been cloned and functionally characterized in humans. These receptors exhibit a variety of physiological actions ranging from cell volume regulation, intercellular communication to apoptosis and proliferation via different signaling pathways. The mechanisms underlying the regulation of their signal transduction are not yet clear. We used HEK 293 cells stably expressing the rat lung P2Y2 receptor (P2Y2-R) tagged to green fluorescent protein (GFP) to investigate the regulation of signaling of the receptor. The P2Y2-R responds equipotently to ATP and UTP. Here we show the visualization of agonist-induced trafficking of the GFP tagged P2Y2-R. The internalization of the P2Y2-R revealed a different concentration-dependent profile compared to the potency of nucleotide induced Ca^{2+} release. A difference in the internalization rate of the receptor was observed upon stimulation of the cells with identical concentrations of ATP or UTP. Differential compartmentalization of the receptor during endocytosis was demonstrated by co-localization with markers for clathrin, early endosomes and lysosomes. P2Y2-R recycling to the plasma membrane could be followed by confocal microscopy and gave full resensitization after 1 hour, as demonstrated by functional Ca^{2+} release. Thus, upon agonist stimulation P2Y2-R signaling is regulated by recycling and degradation of receptors. These results indicate that the signal transduction regulation of the P2Y2-R

depends on the concentration and duration of stimulation. This complex interplay could also be important for nucleotide-mediated regulation of cellular proliferation by P2Y2-R.

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Two novel GABA mimetics reveal functional discrimination on different $GABA_A$ receptor subtypes 824

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γ-Aminobutyric acid type A receptors ($GABA_A$) are responsible for the fast synaptic inhibitory neurotransmission in the mammalian brain. Their structural diversity forms the basis for the functional and pharmacological heterogeneity of GABAergic neurotransmission. The drug therapy of insomnia, anxiety, epilepsy and surgical sedation presently targets most $GABA_A$ receptor subtypes non-selectively, but the heterogeneity of the receptors theoretically holds promise for a brain region- or even cell-selective pharmacological intervention.

In the present study we analyzed the two novel GABA mimetics 5-(4-piperidyl)-1,3,4-oxadiazol-2(3*H*)-one (MJ-315) and 5-(4-piperidyl)-1,3,4-oxadiazol-2(3*H*)-thione (MJ-325) for their ability to differentiate $GABA_A$ receptor subtypes. Starting from N-protected ethyl isonipecotate, the ester was converted to the corresponding acid hydrazide, and subsequently the oxadiazole ring was completed either with carbonyldiimidazole (MJ-315) or carbon disulfide (MJ-325). Deprotection yielded the final test compounds.

In autoradiographics on rat brain cryostat sections MJ-315 and MJ-325 acted like weak partial agonists or antagonists on [^{35}S]t-butylbicyclophosphorothionate ([^{35}S]TBPS) binding to $GABA_A$ receptor channels depending on the brain area. Together with 2 μm GABA these substances potentiate the GABA effect even in those regions where they alone increased [^{35}S]TBPS binding. These findings were supported by our results of the functional biochemical [^{35}S]TBPS binding assay on rat cortical and cerebellar membranes. Furthermore [^{3}H]muscimol binding studies on rat cortical and cerebellar membranes give clear evidence of a low potency GABA agonism of MJ-315 and MJ-325 with K_D-values of several μmol.

Direct functional analysation of MJ-315 and MJ-325 was done with the whole cell configuration of the patch-clamp technique by applying increasing MJ-concentrations with a GABA amount of the EC_{40} on green fluorescenting cells. In order to check subunit specific effects of both compounds, every $GABA_A$ receptor α-subunit was tested for its reactivity to GABA and MJ in combination with the β3 and γ2 subunit. In general,

both substances exhibit clear functional subtype discrimination, and induce alone only in α2, α3, and α5 containing receptors considerable chloride currents.

825 Determined Switch of GABA Sensitivity by Pointmutations in $GABA_A$ Receptors α Subunits

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γ-Aminobutyric acid type A receptors ($GABA_AR$) mediate the fast synaptic inhibitory neurotransmission in the mammalian brain. They belong to the superfamily of ligand-gated ion channels and are heterooligomeric proteins spanning the membrane to form an integral anion-permeable channel. A presently unknown number of different receptor subtypes derives from a pentameric combination of various subunits (α1-6, β1-3, γ1-3, δ1, ε1, θ1, π1, σ1-3), which display a unique pattern of spatial and temporal distribution throughout the CNS. This structural diversity has crucial consequences on the function and the pharmacological properties of native receptors. Distinct alterations in the expression amongst the α subunits is an important feature during development but occurs also under pathological conditions, like epilepsy. This might be due to the fact, that $GABA_AR$ subtypes display an altered sensitivity against their endogenous ligand GABA, depending on the a isoform contributing to the receptor complex.

To pin down the molecular determinants of this phenomenon, we previously created several recombinant chimeric proteins of the (rather insensitive) α3 subunit and the (highly sensitive) α1 subunit. The chimeras displayed characteristics of either the α1 or the α3 subunit and enabled us to narrow the relevant sequences to approximately 70 amino acids. An alignment of these sequence amongst all α subunits revealed a stretch of four consecutive residues which varies between all α isoforms. We changed the four amino acids (ITED in α1 and LVDN in α3, respectively) into the corresponding residues by *in vitro* mutagenesis and analysed the GABA sensitivity of the resulting mutant subunits (α1ITED-LVDN α3 and α3LVDN-ITED α1).

Direct functional analysis of wildtype (wt) and mutant $GABA_A$ receptors were done with the whole cell configuration of the patch-clamp technique on HEK 293 cells cotransfected with expression vectors of β3, γ2 and the desired α subunit. Normalized GABA dose respond curves elicited significant differences in all important characteristics (starting points, EC_{100} and EC_{50} values) for α1- and α3-wt receptors (EC_{50}: 2.87 ± 0.16 μm and 48.84 ± 1.79 μm, respectively). Compared to the α1-wt, α1ITED-LVDN α3 receptors dose respond curve displays identical characteristics to the α3-wt curve (EC_{50}: 53.03 ± 2.14 μm), whereas the properties of the α3LVDN-ITED α1 curve (EC_{50}: 3.2 ± 0.37 μm) reveals high similarity to that of α1-wt.

In order to check the general influence of the four amino acids on the GABA sensitivity of a subunits, the α3 LVDN motif was additionally introduced into the α4 and the α5 subunit. Dose respond curves for GABA changed significantly for α4IMRN-LVDN α3 compared to α4-wt (EC_{50}: 43.20 ± 2.49 μm versus 7.81 ± 0.31 μm) and also for α5LEDD-LVDN α3 compared to α5-wt (EC_{50}: 46.28 ± 2.36 μm versus 11.4 ± 0.68 μm) and were both in the range of the α3 subunit.

We conclude that a stretch of four amino acids in the extracellular N-terminal domain defines the GABA sensitivity of the $GABA_AR$ α subunits. Exchange of these residues in a given subunit by the corresponding amino acids of another α isoform switches the GABA sensitivity in a specific and determined manner.

Capacitive opening of recombinant voltage-gated K^+ channel on silicon chip 826

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To understand and optimize capacitive excitation of neurons from silicon microstructures, it is necessary to study the response of defined voltage-gated ion channels to voltage transients applied to a chip.

HEK 293 cells were stably transfected with Kv1.3 potassium channels. The cells were cultured on silicon chips insulated with a thin dielectric and coated with fibronectin. Voltage transients were applied to the chip. They were chosen such that capacitive coupling gave rise to a stationary voltage in the narrow extracellular space between chip and cell. The resulting changes of potassium current through the attached membrane were recorded at constant intracellular voltage using whole-cell patch clamp.

We succeeded in capacitive gating of Kv1.3 channels through negative extracellular voltages elicited from silicon. The negative extracellular polarization was equivalent to a positive intracellular polarization. Extracellular voltage-clamp with sufficient amplitude and sufficient duration required, however, an enhanced time constant of high pass coupling that was achieved with a high-k dielectric on silicon and a high resistance of the electrolyte.

Distribution and properties of functional postsynaptic kainate receptors on neocortical layer V pyramidal neurons 827

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The distribution of glutamate receptor subtypes on the surface of neurons is highly relevant for synaptic transmission and signal processing. In the present study we investigated the location and properties of functional kainate receptors (KARs) on the somatodendritic membrane of rat neocortical layer V pyramidal neurons. Infrared-guided laser stimulation was used to apply glutamate photolytically to the soma and various sites along the apical dendrite. Electrical currents, resulting from activation of pharmacologically isolated KARs, were measured by whole-cell patch-clamp recording. In addition, KARs on somatic and dendritic outside-out patches were activated while still within the brain tissue.

We found that functional KARs are located on the entire somatodendritic membrane examined. Fast kinetics, a linear *I-V* relationship and a relatively high single-channel conductance are characteristic features of these receptors. We provide evidence that the unitary properties of somatic and dendritic KARs are identical. Regarding the subcellular distribution of KARs, our results indicate that the density of these receptors increases towards the distal dendrite. They are located mainly at extrasynaptic sites, but also mediate fast synaptic signaling triggered by afferent stimulation.

The differential distribution speaks in favor of a selective targeting of KARs on central neurons and may reflect a mechanism for a location-dependent regulation of synaptic efficacy. Furthermore, it is feasible to assume that extrasynaptic KARs could be activated by a 'spillover' of synaptically released glutamate, ambient glutamate in the cerebrospinal fluid, or glutamate released from adjacent astrocytes.

828 Molecular evolution and function of the $GABA_A$ receptor β-4 and γ-4 subunits

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Inhibitory neurotransmission in the vertebrate brain is predominantly mediated by γ-aminobutyric acid (GABA) type A ($GABA_A$) receptors, which are pentameric membrane-associated signalling molecules. In mammals, DNA cloning has revealed the existence of a plethora of receptor subunits (named α1-α6, β1-β3, γ1-γ3, δ, ε, π and θ), each of which is encoded by a separate gene (see 1, 2, and references cited therein). The differential assembly of these polypeptides results in a very large but, as yet, unspecified number of receptor subtypes. It is generally assumed that the different $GABA_A$ subtypes fulfill distinct physiological functions; for example, there is strong evidence that receptors containing the α5 subunit, which is predominantly located in the hippocampus, appear to modulate cognitive processes (3). Results of linkage and radiation hybrid mapping studies in man have shown that most of the $GABA_A$ receptor subunit genes are located in four clusters, on chromosomes 4, 5, 15, and the X (4). It is assumed that these clusters originated from an ancestral cluster of three genes (an α-like, a β-like and a γ-like) via two genome duplications (tetraploidisation events) that occurred during chordate evolution. Interestingly, certain vertebrate classes (for example, birds and reptiles) appear to possess two unique $GABA_A$ receptor genes that encode subunits that have been named β4 and a γ4. It has been suggested that these replace the mammalian β1 and γ3 subunits, which have not been found in lower vertebrates (1). The γ4 subunit, which has been studied in detail, is extant in *Gallus domesticus* (chicken), *Taeniopygia guttata* (zebra finch), *Serinus canaria* (canary) and *Trachemys scripta elegans* (turtle; C. Thode & M. G. Darlison, manuscript in preparation). Furthermore, receptors containing this polypeptide have been implicated in the process of imprinting in chicks, which is a form of recognition memory (5). In contrast, comparatively little is known about the β4 subunit. In this presentation, we will describe the isolation and sequences of β4-subunit complementary DNAs from a variety of vertebrate species. These data permit an insight into the evolution of this important polypeptide, which is known to form homo-

oligomeric GABA-gated chloride channels (6). Finally, we will present comparative data on the distributions, in brain, of the β4-subunit transcript.

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Actin filaments modulate voltage-gated calcium channels in retinal ganglion cells

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Polymerization and disruption of actin filaments participate in many aspects of activity-dependent synaptic plasticity. Actin filament networks interact with ion channels and neurotransmitter receptors, and shape post- and presynaptic components of the synapse. In the vertebrate retina, intracellular calcium ($[Ca^{2+}]_i$) regulates the activity of voltage-, and ligand-gated ion channels (Akopian and Witkovsky, 2002, Mol Neurobiol 25:113). Evidence indicates that some regulatory mechanisms of $[Ca^{2+}]_i$ may indeed be mediated through changes in the dynamics of the actin cytoskeleton (Nakamura et al., 2000, Am J Physiol Cell Physiol 279:480).

We therefore investigated the effects of actin filament disruption on the functional activity of voltage gated calcium channels in salamander retinal ganglion cells. Neurons were enzymatically isolated from the retinas of tiger salamanders (Ambystoma tigrinum). Cells were incubated in Ringer solution containing 30 μm Fura-2 AM for 40–50 min. Changes of $[Ca^{2+}]$i were monitored with a PTI calcium imaging system using 340 nm for excitation. The fluorescence intensity (monitored at 510 nm) within a cell was normalized to its resting level and expressed as the ratio $\Delta F(t) = [F(t)-F]/F$, where F(t) is the intensity of the fluorescence at time t, and F is the averaged fluorescence intensity of a 15 sec baseline period. We monitored putative ganglion cells (soma > 15 μm) which retained a portion of their axon and short dendritic processes. In parallel experiments, we patch clamped ganglion cells in retinal slice preparations and recorded calcium currents in the whole cell voltage clamp mode. To isolate calcium currents, the measurements were carried out in the presence of TTX (1 μm) and TEA (20 mM). Selective actin depolymerising drugs, cytochalasin B (20 μm) and latrunculin B (20 μm) were added to the patch pipette solution, while a membrane permeable analog, latrunculin A (5 μm) was applied in external solution during the calcium imaging experiments.

Calcium signals induced by high-K^+ (100 µm) application were measured before and after 10, 30 and 60 min of latrunculin A incubation. The amplitude of $[Ca^{2+}]_i$ signals was significantly reduced after 30 min (82 ± 10 %) of latrunculin A treatment (n=3). There was no significant change in $[Ca^{2+}]_i$ in the control situation during the same time period (n=4). Whole-cell calcium currents were recorded by applying depolarizing pulses from –60 to +60 mV (20 mV increments, V_{hold} = -70 mV). In some experiments, calcium currents were elicited by a voltage ramp from –70 to +70 mV (200 ms). Currents were measured within 1 min of patch rupture and after 15-30 min incubation with the actin depolymerising drugs. The mean reduction of calcium currents after filament disruption was 45 ± 4 % (n=7). Sodium currents measured under identical conditions were not reduced.

These data indicate that disruption of actin filaments results in a decrease of voltage gated calcium channel activity. Since $[Ca^{2+}]_i$ regulates a variety of processes linked to synaptic efficiency, the dynamics of the actin network is an essential component of synaptic interaction.

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830 Automated patch-clamping with the novel CytoPatch™ technology

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The knowledge about structure, function and regulation of ion channels and their involvement in various diseases has strongly increased. As a consequence, ion channels receive special attention as a target class for drug discovery by the pharmaceutical industry. To meet the needs of industrialized drug discovery adequate methods and instrumentations are required for screening a large number of compounds against this target class.

Ion channels, however are a target class hard to exploit, because reliable recording of ionic currents under a defined membrane voltage can only be obtained by the patch-clamp technique. However, this technique has not been available for screening due to its very low throughput. Devices for automated patch-clamping will overcome this limitation.

Patch-clamp automates for whole-cell recordings must (1) bring isolated cells in contact with patch contacts, (2) form gigaseals and (3) establish stable intracellular access that allows for high-quality recording of ionic currents. To settle this three tasks with a micromachined patch contact we developed the so-called CYTOCENTERING

technique to apply to suspended cells the same operation sequence as in conventional patch-clamping. It combines the advantage of openings with diameter larger than 4 µm in planar substrates for attraction of suspended cells by moderate suction and pipette openings smaller than 2 µm for seal formation.With this method we immobilized selected cells from a flowing suspension on the tip of a patch pipette by suction with a success rate of 97 % and formed gigaseals with a success rate of 68 %. Subsequent whole-cell recordings and intracellular staining with Lucifer Yellow proved the stable access to the cytoplasm.

A micro-structured glass chip with embedded, concentric arranged suction and contact openings has been fabricated. This CYTOPATCH^{TM} chip replaces the patch-pipette and all means for cell positioning. It allows to apply the same protocols for whole-cell analysis as the conventional whole-cell patch clamping does - with the same quality and information content.

The CYTOPATCH^{TM} chip is a disposable that is reloaded automatically for each cell into the CYTOPATCH^{TM} automat. For higher throughput (up to 10 000 cells per day, depending on the performed patch clamp protocol) the automat can operate multiple CYTOPATCH^{TM} chips, individually and fully automated handled for asynchronous recording of up to 50 parallel-patched cells.

Sodium-dependent potassium currents in lamprey spinal neurons: Effects of replacement of extracellular sodium by lithium

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Several types of sodium-dependent potassium currents (K_{Na}) have been described both in vertebrates and invertebrates. To reveal these currents substitution of extracellular Na^+ with Li^+ was used as a standard method. Li^+ is able to pass through voltage-gated Na^+ channels, but is not supposed to activate sodium-dependent channels, like K_{Na} channels. Here the types of K_{Na} and their role have been examined in lamprey spinal neurons. In addition we focused on the further consequences of extracellular replacement of Na^+ by Li^+.

Whole-cell voltage-clamp measurements were made from dissociated spinal neurons. In all neurons recorded depolarisation of the membrane potential from -60 mV to -10 mV elicited an inward Na^+ current followed by a fast transient and a slow sustained K^+ current. Application of TTX (600 nM) did not only block the Na^+ current, but also abolished the transient K^+ current, suggesting that it corresponds to a transient K_{Na} current. Li^+ carried an inward current that was slower than that carried by Na^+ and the activation curve of the Li^+ carried current was shifted towards more positive values (+7 mV), while the inactivation curve was not affected. The transient K_{Na} current persisted when Na^+ was replaced with Li^+ while the sustained K^+ current was significantly reduced. These data suggests that Li^+ can activate a transient current comparable with the transient K_{Na} and that a part of the sustained current is mediated by activation of a slow K_{Na} current. A

similar decrease in the sustained current was obtained when Na^+ was replaced with NMDG or choline.

The effect of substitution of Na^+ with Li^+ on the firing properties was examined in current clamped spinal neurons. At low stimulus intensity (0.3-0.5 nA), the number of action potentials was reduced, while no change was seen at high stimulus intensity (0.7-1 nA). The decrease in the firing induced by Li^+ was due to the shift of the activation of the Li^+ carried inward current. In the intact spinal cord, replacement of Na^+ with Li^+ decreased the amplitude of the non-K_{Ca} dependent AHP, suggesting that it is mediated by the slow sustained K_{Na} current. The fictive swimming rhythm induced by NMDA in the intact spinal cord was slowed down when Na^+ was replaced with Li^+. This could be due to the change in the AHP amplitude and also to a decrease of NMDA-induced current in the presence of Li^+.

In conclusion, we have shown that lamprey spinal neurons possess a transient K_{Na} current activated by Na^+ influx during action potentials that play a role in controlling the early repolarization phase of action potentials. In addition, there is a slow K_{Na} current, which might underlie the slow non-K_{Ca} AHP to regulate the firing of neurons in the locomotor network.

832 Ionotropic receptors of cultured honeybee antennal lobe neurons

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The antennal lobes constitute the primary olfactory neuropile in the insect brain. First-order interneurons form a network that produces complex spatio-temporal patterns of odor-induced activity. Immunocytochemistry indicates the expression of acetylcholine (ACh), GABA, histamine and glutamate receptors in antennal lobe neurons (review: Homberg, 2002, Microsc Res Tech, 56(3):189-209), but functional ionotropic receptors have not yet been physiologically identified. We, therefore, investigated the presence and properties of ionotropic receptors on cultured antennal lobe neurons of the honeybee, *Apis mellifera*, using the whole-cell configuration of the patch-clamp technique.

Pressure applied ACh (1mM) induced inward currents with peak amplitudes ranging from -150 to -2050 pA (mean -826.1 ± 70pA, SEM, n=67, pulse potential -110mV). Epibatidine and nicotine acted as only partial nicotinic agonists of the antennal lobe ACh receptors. Bath applications of α-bungarotoxin (1μM) blocked 72 ± 9.9% (n=4) of the ACh-induced current. The potent vertebrate neuronal nicotinic blockers dehydroxy-β-erythroidine (1μM) and methyllycaconitine (100nM) blocked completely and reversibly the ACh-induced current. These results demonstrate a nicotinic pharmacological profile of the ACh receptor in antennal lobe neurons.

GABA (500µM), induced an inward current with peak amplitudes ranging between -40 to -2400pA (mean peak -467.63 ± 94.2pA, n=37, pulse potential -110mV). The GABA-induced current was sensitive to bath applied picrotoxin (100µM; block: 53% ± 13% of the maximum peak current amplitude, n=6). Glutamate (500µM) induced a small inward current (mean peak amplitude -153.4pA ± 20.1pA, n=31). Glutamate currents comprised a rapidly activating and desensitizing and a slowly desensitizing component. Bath application of 100µM picrotoxin blocked reversibly the transient component of the glutamate-induced current. Pulses of glutamate applied at different membrane potentials (-80 to +30 mV) indicated a reversal potential between -40mV and -20mV. Pressure application of AMPA (1mM) did not induce any current. Our data indicate a putative inhibitory glutamate receptor with a chloride permeability that parallels the inhibitory action of GABAergic neurons within the antennal lobe. Bath applications of the insecticide Fipronil (1µM), a known blocker of the GABA receptor, almost completely blocked GABA or glutamate currents, but weakly blocked ACh currents.

Histamine applications (100µM-1mM) did not induce any current on antennal lobe neurons. However, bath applied histamine reversibly blocked 48.4 ± 7% (n=10) of the glutamate-induced current and 17± 8.8% (n=20) of the ACh current. Bath application of histamine (1mM) had no effect on currents induced GABA or on voltage-sensitive currents. The histaminergic antagonist, cimetidine (1mM), blocked reversibly 77.5 ± 7.3% (n=13) of the ACh currents and 74.5 ± 7.3% (n=7) of the glutamate-induced currents, but had no effect on the GABA-induced currents.

We identified functional ionotropic GABA and glutamate receptors, which may provide inhibitory pathways. The nicotinic ACh receptor of the antennal lobe neurons, like its counterpart in the honeybee Kenyon cells (Goldberg, Grünewald, Rosenboom & Menzel, 1999, J Physiol 514.3:759), probably mediates fast excitatory synaptic transmission. We found no evidence for ionotropic histamine receptors, but histamine may act as an inhibitory modulator of the ionotropic glutamate receptor.

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Potassium channel KCNQ4 833

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The potassium channel KCNQ4 has been described to be expressed in outer hair cells of the mammalian cochlea and is linked to nonsyndromic dominant deafness DFNA2 (Kharkovets et al., 2000, PNAS 97). In mature cochlear outer hair cells KCNQ4 is localized in the basal part of the cell and is presumed to be responsible for the repolarisation of the outer hair cells. We noted an alteration of the subcellular distribution of KCNQ4 coincident to an alteration of the subcellular distribution of the outer hair cell motor

protein prestin prior to the onset of hearing. As the prestin expression itself as well as its subcellular distribution revealed as being under control of thyroid hormone (TH) (Weber et al., 2002, PNAS 99), we analysed the effect of TH on KCNQ4 expression in hypothyroid mice animal models, in which TH was either suppressed upon a point mutation in the thyrotrophin receptor (Tshr mutant mice; Walsh and McGee, 2001) or upon thyroid agenesis (Pax8 mutant mice; Mansouri et al., 1998, Nat.Genet. 19), as well as in euthyroid and hypothyroid TH-receptor (TR) mutant mice, (TRalpha1-/- and TRbeta-/-). Suprisingly we noted TH to control the transcription of both KCNQ4 and prestin, acting via derepression of TRalpha1 on KCNQ4 but via enhancement on prestin. In line with this strong effect on KCNQ4, putative TH response elements (TREs) were identified in the genomic upstream region of KCNQ4. A novel role of TH for membrane targeting could be shown for prestin, presumptively mediated through TRbeta. Data may elucidate a differential role of TH for the development of a final cellular phenotype and indicate to a so far undescribed role of TH for protein targeting.

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834 Transcriptional control of the cochlear motor protein prestin

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Electrical stimulation during the hearing process induces rapid length changes of cochlear outer hair cells. The molecular motor (prestin) has recently been identified (Zheng, J. et al. 2000 Nature 405, 149-155). In our effort to find transcriptional regulators of the prestin gene we have identified a functional Thyroid Hormone Response Element (PresTRE) at position -416 in relation to the ATG codon of rat prestin (Weber, T. et al. Knipper 2002 PNAS 99, 2901-2906). We determined the start site of transcription in the rat using RACE-PCR. After alignment of the RACE sequence data with the homologous human genomic sequence we found that PresTRE is located downstream from the startpoint of transcription, probably in the second intron. We therefore started to analyse up to 1 kb upstream of the transcriptional start point of the human prestin gene using computer programmes. We were able to note a number of putative regulatory elements which may belong to the minimal prestin promotor. Fragments differing in their length from within these first 1 kb have been cloned. First studies analysing specifity and function of distinct binding sites in this region will be presented and discussed in the context of their function for the control of prestin gene expression.

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Differential transcriptional control of cochlear ion channels dependent on the onset of expression 835

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Retardation of the expression of the fast-activating potassium channel IK,f or BK-channel was recently documented to be related with deafness in thyroid hormone receptor β mutant mice (Ruesch et al., 1998, PNAS 95). In the developing cochlea various ion channels are expressed in outer and inner hair cells as well as spiral ganglia neurons far beyond, shortly before or during the onset of hearing. We analysed the expression of different ion channels (Kv, Kir4.1, KCNQ4, BK, SK2, AchRalpha9/10, Prestin,) in hair cells and ganglia neurons and studied a presumptive thyroid hormone dependency in animals in which TH is retracted by goitrogen, by TSH receptor mutation or TR mutations. Data may hint to a new principle by which TH affects phenotypically important genes in the inner ear.

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ATP induces cell permeabilization in the intact rat retina 836

Innocenti Barbara, Sylke Pfeiffer, Konrad Kohler, Elke Guenther and Eberhart Zrenner

The $P2X_7$ receptor belongs to a family of cation channels that are activated by ATP. Different from other P2X receptors, prolonged activation of $P2X_7$ can result in an additional opening of a membrane pore that is permeable to molecules up to 900 Da and has been shown to induce cell death in the immune system.

We have previously reported that $P2X_7$ receptors are expressed in the rat retina and located in the inner nuclear and ganglion cell layer (GCL). The present study was aimed to investigate if the $P2X_7$ receptor can act as a mediator of cell permeabilization in the rat retina and to identify the putative cellular target(s) of its effect.

We used a fluorescence assay to assess whether $P2X_7$ receptors were functional in flat-mounted retinas isolated from Brown Norway rats. As an indicator of cell permeabilization we used the fluorescent dye YO-PRO-1 (MW 375Da). The dye enters into the cells through large pores like those opened by prolonged or sustained stimulations of the $P2X_7$ subunit. After entering a cell, YO-PRO-1 binds to DNA, thus providing a stable labelling of the activated cells.

Different agonists for P2 receptors (ATP, BzATP, ATPgammaS, ADP and UTP) were tested for their ability to cause cell permeabilization in the inner rat retina. Among them, only high concentrations of ATP (500μM) and BzATP (100μM) were able to induce

accumulation of YO-PRO-1 in the inner retina. This effect was blocked by the P2-antagonists suramin and PPADS (100µM). The two set of experiments together supported the hypothesis that cell permeabilization in the retina can be induced by activation of the $P2X_7$ subunit.

The fluorescence signal was detected in the GCL and in the nerve fiber layer, suggesting that different cell types were responding to $P2X_7$ stimulation. In order to identifying the retinal cell types affected by ATP-induced permeabilization, we used *'in vivo'* labelling techniques. The YOPRO-1 uptake assay was performed on whole mount retinas where ganglion cells, microglial, Müller or other glial cells, one cell type at a time, were labelled with specific markers. We were thus able to identify microglial cells as targets of $P2X_7$ induced cell permeabilization.

Despite the strong expression of $P2X_7$ subunit by different cell types in the inner rat retina, prolonged stimulation of this subunit elicits permeabilization only in microglial cells.

The different activation properties in different retinal cell types suggests that $P2X_7$ is involved in multiple physiological and pathological states of the mammalian retina.

837 A stochastic gate model for the transduction channel of cochlear hair cells

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Compared to voltage-gated and ligand-gated ion channels the mechanosensitive ion channel in the mammalian cochlea reveals much faster gating up to 120 kHz. It transforms sound into an electrical receptor current with sensitivity to forces in the pN-range. Mechanoelectrical transduction was studied so far investigated deflecting the entire hair bundle of cochlear sensory cells rather than individual stereocilia. Here we present a study displacing single tallest stereocilia of living outer hair cells of postnatal rats while the current response is measured simultaneously by patch clamp. The saturated transduction channel reveals current amplitudes of 7 pA to 84 pA. Géléoc et al. (1997) could show in their experiments that the single channel amplitude of an outer hair cell mechanoelectrical transducer channel in mice is about 10 pA. According to this, a displacement of individual stereocilia in our experiments supposes an opening of about 1 to 8 transduction channels. Surprisingly, we also found non-saturated transduction current responses of 2 pA to 3 pA following directly the mechanical stimulus. The common single channel gating hypothesis is not sufficient to explain these data, assuming a single channel amplitude of 10 pA. Different models, including a novel stochastic gate model, predicting a high frequency flickering of the transduction channel will be discussed.

Probing the structure of mechanosensors in the inner ear by Scanning Probe Microscopy using a novel experiment control and automation software

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The cochlea is the organ of hearing in mammals and embeds the organ of Corti with the mechanosensitive cells ? the hair cells. The rod-like stereocilia located on top of the hair cells are graded in height and transduce mechanical stimuli into electrical signals by the so-called mechanoelectrical transduction channel. The rows of stereocilia are connected by tip links, which are thought to be directly connected to the transduction channel. Since there is less known about the gating mechanism of these channels a new technique was required. We have introduced the scanning probe microscopy (SPM) to hearing research to investigate the structure-function relationship of mechanosensitive structures under physiological conditions. For a more specific nanomanipulation of a single stereocilium an advanced setup and especially a powerful and flexible software is needed. Such a software must allow the sequential execution of different measurement procedures. Between these procedures it should be able to set control parameters based on results of previous measurements. In our case, the localization and manipulation of a stereocilium, the hair cell bundle has to be scanned and visualized. Afterwards the stereocilium has to be selected within the image in order to apply a manipulation procedure to it. While conventional software products are often highly specialized applications for a particular use such as patch clamp for instance, we have developed a new experiment control and automation software: *ScanClamp*. It supports arbitrary definition of measurement protocols and some 2D/3D visualization modes. Thereby not only precise control of the SPM setup but also control of electrophysiology equipment like patch clamp can be achieved in the same software environment. Even if only a few instruments are supported so far, the open architecture should allow easy integration of further instruments. In cooperation with Kleindiek Nanotechnik also a new SPM hardware has been developed.

For further information: *Division of Sensory Biophysics*: http://thrc.hno.medizin.uni-tuebingen.de/groups/langer *ScanClamp*: http://thrc.hno.medizin.uni-tuebingen.de/scanclamp *Kleindiek Nanotechnik:*: http://www.nanotechnik.com

839 Identification and Characterization of Novel Interactionpartners of the Inhibitory Glycine Receptor Subunit Alpha 2

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Glycine receptors are the major inhibitory neurotransmitter receptors in spinal cord and brain stem. The receptor consists of two β and three α subunits. In higher animals four different isoforms of the a subunit were identified, which are able to form functional homomeric receptors in heterologous systems. The α 1 and α 3 subunits are mainly expressed in adult organisms in spinal cord and cerebellum respectively, on contrast to the GlyR α 2 which was postulated to be the embryonic isoform. The role of α 4 is not yet clarified.

Recently it was shown that there is still functional α 2 present in certain forebrain regions like amygdala in adult animals despite the fact that the expression level of α 2 sharply declines three weeks after birth. In addition in some nonneuronal adult tissues GlyR's were identified.

To investigate the role of α 2 subunit in the development of inhibitory synapses and the regulation of the receptor assembly, a biochemical approach was used to identify interacting proteins with GlyR α 2. For this purpose the cytoplasmic loop between M3 an M4 was expressed as GST-fusion protein. The immobilized fusion protein was incubated with extracts from different CNS regions and developmental stages and small set of proteins were identified by MALDI-MS to be specifically bound and eluted from α 2 loop fusion proteins. Currently cellular and biochemical approaches are used to verify the specific interaction with the α 2-subunit and to investigate the physiological role of this protein-protein interactions.

840 Splicevariants of the NR1-subunit of the NMDA-receptor are differentially regulated by receptor activity during synaptopgenesis of rat embryonic spinal cord neurons *in vitro*

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NMDA-receptors play important roles during synaptogenesis, i.e. synapse formation and synaptic plasticity. The receptor is composed of NR1- and NR2-subunits. Whereas the known NR2-subunits (NR2A-D) are encoded by four different genes, eight possible splicevariants of the NR1-subunit are encoded by one gene. Evidence from the last years pointed to important roles of the NR1-splicevariants for (1) receptor clustering and composition, (2) synaptic transmission and (3) effects on intracellular signaling pathways.

We investigated the expression of the two different NR1-C-terminal splicevariants (C2 and C2?) using immunofluorescence and confocal microscopy during synaptogenesis of rat embryonic spinal cord neurons *in vitro* and examined the localization of these splice-variants at extra- and postsynaptic sites. Moreover we focused on wether or not the expression and localization is dependent on receptor-activity by using chronical administration of NMDA-receptor agonist (NMDA) and antagonist (MK801).

Our results show that at early developmental stages (days *in vitro*; DIV; DIV8) both, C2 and C2?-C-terminal cassette containing NR1-subunits are expressed. Of this, the C2 is the prevailing variant, with an expression at extrasynaptic and postsynaptic sites as revealed by double-labeling with the presynaptic vesicle protein synaptophysin. By altering NMDA-receptor activity a different regulation of both C-terminal cassettes is evident.

At DIV22, NMDA-receptor inhibition leads to a strong increase in the number and the postsynaptic localization of the C2?-cassette. In addition, a chronic receptor activation by NMDA led to a decrease of C2?-puncta, suggesting that postsynaptic anchoring of C2?-containing NR1-subunits is negatively regulated by receptor activity. In contrast to this the use of a C2-specific antibody did not reveal a drastic change of postsynaptic cluster number by receptor activity.

Thus, our results show a differential expression of the NR1-C-terminal splicevariants C2 and C2?, with respect to (1) the developmental regulation and (2) the expression pattern at extra-and postsynaptic sites. Moreover, our results clearly indicate for the first time that NMDA-receptor activity regulates NR1-splicevariants differentially. Further studies are now in progress in order to analyze the cellular mechanisms underlying this differential regulation of NMDA-receptor-cluster formation.

Expression of a soluble glycine binding domain of the n-methyl-d-aspartate receptor NR1 subunit

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Glycine is an essential co-agonist of the excitatory N-methyl-D-aspartate (NMDA) receptor, a subtype of the ionotropic glutamate receptor family. The glycine binding site of this hetero-oligomeric ion channel is formed by two distinct extracellular regions, S1 and S2, of the NR1 subunit. Here we report the production and purification of different S1S2-fusion proteins in E.coli. This fusion proteins are tagged with a His6 epitope, and two different linkers of 13 or 2 amino acids are connecting S1- and S2- segments of various length, in order to determine the minimal sequence regions, essential for the formation of a functional glycine binding site.

In addition, we have established a procedure for the renaturation of denaturated S1S2-proteins isolated from inclusion bodies. After renaturation and purification the fusion proteins bound the competitive glycine site antagonist [3H]MDL105,519 with different affinities, in some cases similar to that determined with rat brain membrane fractions. Using "half-native" SDS-gel electrophoresis and immunoblot analysis we could show

that the S1S2-protein variants differ in respect to their aggregation state (e.g monomeric, dimeric or high molecular aggregates). These studies should allow to optimize the expression of S1S2-glycine binding sites in order to achieve a high level of correctly folded protein for further structural analyses.

842 Pharmacological discrimination of somatic and prejunctional nicotinic acetylcholine receptor(nAChR)channels in the mouse superior cervical ganglion(SCG)

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Neuronal-type nAChRs play a pivotal role in the trans-ganglionic signal transduction of the vegetative nervous system. Two ganglia have previously served as model structures to study the properties of these receptors: the cholinergic (chick) ciliary ganglion, and the adrenergic rodent superior cervical ganglion (SCG). In addition to somatodendritic nAChR's located within the SCG, these neurons have also prejunctional receptors on their nerve endings in target organs, where nicotinic agonists upon infusion may generate antidromic volleys and cause the release of norepinephrine.

The primary subunits that make up peripheral nervous system nAChRs are $\alpha 3$ and $\beta 4$. However, RT-PCR experiments and the binding of α bungarotoxin suggest the presence of (accessory) $\alpha 5$, $\alpha 7$, and $\beta 2$ subunits in the rodent SCG that by their inclusion might modulate the function of $\alpha 3/\beta 4$ receptors. We have previously shown (Kristufek et al., J. Physiol. 516, 739 - 756, 1999) that somatic receptors in cultured rat SCG neurons (assessed with patch clamp recordings) are more potently activated by cytisine, whereas prejunctional receptors (assayed by the release of [3H] norepinephrine) are more sensitive to DMPP.

However, our recent experiments on both rat and mouse SCG indicate that the agonist profiles for somatic, but not prejunctional, nAChRs change by the age of cultures. In order to test the hypothesis that the observed phenomena are due to a differential expression of accessory subunits at somatic and axonal domains we extended the experiments to mice with functional deletions of $\alpha 5$, $\alpha 7$, and $\beta 2$ subunits. Initial observations using SCG cultures from $\alpha 5$ knockout mice suggest pronounced differences to their wild type counterparts in both somatic and prejunctional nAChRs. Accordingly, the efficacy of prejunctional receptors is about twofold in $\alpha 5$-knockout mice compared to wild type controls, whereas the pharmacological profile of somatic receptors in SCGs dissected from knockouts becomes independent of the age of the cultures. Pending experiments with $\alpha 7$ and $\beta 2$ knockout mice will show the contribution of these subunits to the function of somatic and prejunctional nAChRs in the mouse SCG.

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Evidence for a subsynaptic pool of $GABA_A$ receptors. 843

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The development of tolerance to benzodiazepine treatment has been suggested to involve $GABA_A$ receptor internalisation. Here we have compared the distribution of cell-surface $GABA_A$ receptors and internalised receptors in hippocampal neurons grown for 2-3 weeks *in vitro*. Living neurons were incubated with an antibody against the α1 or α2 $GABA_A$ receptor subunit and antibody-bound receptors were allowed to internalise. Membrane receptors were visualised by immunofluorescence in fixed, intact neurons, and the internalised receptors were labelled with a different fluorochrome after permeabilisation.

Under these conditions, the membrane receptor staining for the α1 and α2 $GABA_A$ receptor subunit was distributed homogenously over cell body and dendrites, whereas the internalised receptors formed clusters with a characteristic postsynaptic pattern. These clusters were indeed colocalised with gephyrin and apposed to presynaptic terminals. The clusters were only seen when antibody-bound receptors were allowed to internalise at 37°C. The staining intensity of these clusters was time-dependent.

To verify that this pattern corresponds to the existence of a subsynaptic pool the following control experiments were done:

1. No difference in surface staining was observed when the cells were fixed with paraformaldehyde (without permeabilisation) or fixed and permeabilised with methanol, immediately after saturation of surface receptors with antibody. Therefore, steric hindrance that would prevent secondary antibodies from entering the synaptic cleft can be ruled out as an explanation for the difference between membrane and internal staining.

2. Surface receptor-bound antibody was removed using a 30 second acid strip. After this treatment the surface staining had disappeared, whereas the internalised receptor clusters were still detectable.

3. A hypertonic sucrose solution was able to block receptor internalisation, pointing towards the involvement of clathrin-coated vesicle endocytosis.

5. Saturating membrane receptors with a guinea pig α1 subunit antibody under conditions non-permissive for internalisation and consecutive labelling of internal receptors with a rabbit α1subunit antibody revealed the same differential distribution as seen following internalisation of labelled cell-surface receptors. This suggests that there is already a subsynaptic pool of $GABA_A$ receptors present in these hippocampal cultures.

Altogether, these results suggest that at postsynaptic sites, the majority of $GABA_A$ receptors are not actually in the membrane, but are located in an intracellular compartment and colocalised with gephyrin. In conclusion we propose that postsynaptic α1 and α2 $GABA_A$ receptors can shuffle rapidly between the membrane and a subsynaptic compartment containing gephyrin, suggesting the possibility of fast regulation of receptors for neurotransmission, which might be influenced by diazepam.

844 Chronic cannabinoid treatment during puberty leads to disruption in sensorimotor gating, object recognition memory and the performance in a progressive ratio schedule in adult rats

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There is evidence from studies in humans and animals that a vulnerable period to chronic cannabinoid administration exists during certain phases of development. Therefore, the present study tested the hypothesis that chronic interference by cannabinoids with the endogenous cannabinoid system causes specific and persistent behavioural alterations in adult rats only after treatment during peripubertal development.

The chronic treatment of WIN 55,212-2 (WIN) (1.2 mg/kg) or vehicle was extended over 25 days either throughout the rats puberty (postnatally day (pd) 40 - 65) or for a similar time period in adult rats (pd > 70). The rats received 20 injections intraperitoneally (i.p.) which were not delivered regularly. After chronic treatment there was a rest period for all animals for 10 days before behavioural testing was started.

The adult rats were tested for object recognition memory, performance in a progressive ratio (PR) operant behavior task and on prepulse inhibition (PPI) of the acoustic startle response (ASR), an operational measure for sensorimotor gating.

PR test was performed between day 5 and 15 after chronic WIN treatment. PPI was tested on day 20, 55 and again after day 85 following a single i.p. injection of vehicle/haloperidol. The object recognition test was conducted between day 20 and 25 after cannabinoid treatment.

PPI was significantly disrupted only by chronic peripubertal cannabinoid treatment on pd 85, 120 and still on pd 150. This longlasting PPI deficit was reversed by the acute administration of the dopamine antagonist haloperidol after pd 150. Furthermore, we found deficits in recognition memory of pubertal treated rats and these animals showed lower break points in a PR schedule. Adult chronic cannabinoid treatment had no effect on PPI, object recognition and the performance in a PR schedule of reinforcement.

Therefore, we conclude that puberty in rats is a vulnerable period to cannabinoid treatment. The endogenous cannabinoid system seems to be highly susceptible to cannabinoid administration during this developmental phase, as evidenced by the disruption of sensorimotor integration, mnemonic and motivational processes after pubertal cannabinoid treatment.

There is evidence from recent studies for a connection between schizophrenia and cannabis use. Since PPI and object recognition memory are impaired in schizophrenic patients and since the reduction in effort an animal is willing to make in order to obtain a reward on a PR schedule might serve as an animal model for anhedonia, we propose chronic cannabinoid administration during pubertal development as an animal model for at least some aspects of schizophrenia. Evidence for this assumption is also given by the

fact that the cannabinoid-induced PPI deficit observed in our study was reversed by a clinically potent antipsychotic drug.

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Attentional modulation of prepulse inhibition of the acoustic startle reflex in rats with a combined PPI/ conditioned inhibition paradigm

845

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Prepulse inhibition (PPI) of the startle reflex occurs when a sudden, non-startling tactile, visual or acoustic stimuli is presented shortly prior to the startling stimulus and is used as an operational measure for sensorimotor gating mechanisms. PPI is described as an automatic, preattentive phenomenon. However, previous studies have shown that in humans PPI is enhanced when the subjects attended to the prepulse (Filion et al., 1993; DelPezzo et al., 1980).

The aim of the present study was to investigate the possibility of attentional modulation of PPI in rodents and if so, how PPI is affected by this attentional mechanisms.

Male Wistar rats were tested in a set of identical automated startle chambers using different stimulus frequencies, intensities and interstimulus intervals (ISI). For attentional modulation of startle inhibition a combined PPI/ conditioned inhibition paradigm (CIP) was used. Based on preliminary experiments the conditioned stimulus (CS) for fear conditioning (paired with the US) was a 300ms, 6 kHz, 60 dB SPL tone. The conditioned inhibitor (CI), predicting that the CS is not followed by a footshock, was a 300ms, 12 kHz, 60 dB SPL tone. The CS, CI and the compound, where the CI preceded the CS, were presented as a prepulse in a following PPI test.

After conditioned inhibition training, the compound stimulus (CS follows the CI without being paired with a footshock) as a prepulse has the ability to increase the PPI of the acoustic startle reflex compared to untrained rats. In contrast, the CS and CI as prepulses do not affect PPI in a significant manner.

These findings lead to the conclusion, that PPI of the acoustic startle reflex in rats depends on attentional modulations like in humans. This indicates that the PPI of the acoustic startle reflex is more than a pure sensorimotor gate that facilitate attention, but is also under the control of top-down attentional modulations.

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846 Clozapine increases disruption of prepulse inhibition after sustained PCP or MK-801 treatment

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Sustained exposure of non-competitive NMDA antagonists, such as phencyclidine (PCP) or dizocilpine (MK-801), have previously been found to cause progressive enhancement of behavioral abnormalities that may be similar to neuropsychological deficits seen in schizophrenia. In fact, previous studies show that repeated administration of the non-competitive NMDA antagonist MK-801 induces a sensitization of prepulse inhibition (PPI) deficit in Sprague-Dawley rats. The phenomenon of PPI of the acoustic startle response is used as a measure of sensorimotor gating and is reduced in schizophrenic patients. The aim of this study was to induce sensitization of the acute disruptive effect of PCP and MK-801 on PPI by repeated administration and to assess the effects of the atypical antipsychotic compound clozapine on sensitization.

Over a period of 11 days male Wistar rats received daily subcutaneous injections of either PCP (2mg/kg, n=10), MK-801 (0,1mg/kg, n=9) or saline (1ml/kg, n=9) before testing of PPI in a startle response system. After this pretreatment all groups were challenged with PCP alone and in combination with 5 or 10mg/kg clozapine prior to testing procedure. Finally, all groups received 5mg/kg clozapine only. Between experiments there was at least one day without treatment.

Injections of PCP and MK-801 induced a significant disruption of PPI ($p<0.05$) but this effect did not increase over days. PCP challenge alone induced a PPI deficit in all groups regardless of pretreatment with PCP, MK-801 or saline ($p<0.05$). However, a combined treatment with PCP and 5mg/kg clozapine induced a PPI deficit that was further enhanced in PCP and MK-801 pretreated rats compared to PCP challenge alone ($p<0.05$). PPI deficit in saline pretreated rats was not additionally affected. Finally, treatment with clozapine alone induced a PPI deficit in PCP pretreated rats compared to saline pretreated rats ($p<0.05$).

Repeated administration of NMDA receptor antagonists did not induce sensitization of PPI deficits in Wistar rats. Surprisingly, the antipsychotic clozapine enhances the PPI disruptive effect of PCP and MK-801 pretreatment.

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847 Effects of neonatal medial prefrontal cortex lesions on trace fear conditioning in rats

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In classical (delay) conditioning the conditioned stimulus (CS) and the unconditioned stimulus (US) are usually presented in a temporally contingent manner. If this temporal

relationship is not given because a stimulus-free interval separates CS and US (trace conditioning) it is more difficult for an animal to form associations between these stimuli. It is known that in this case the medial prefrontal cortex (mPFC) plays an important role and that adult lesions of the mPFC lead to impairments in the ability to learn trace-conditioning tasks. The aim of the present study was to investigate whether neonatal lesions of the mPFC lead to deficits comparable to or worse than adult lesions or if an early developmental damage can be compensated.

To induce a mPFC lesion male Wistar rats were bilaterally injected with ibotenic acid (0,2 µg / 0,3 µl / hemisphere) or 0,3 µl tris-buffered saline / hemisphere on postnatal day 7 or at an adult stage. 16 weeks (for the neonatal group) and 4 weeks (for the adult group) after lesioning animals were trained in a fear conditioning paradigm with a 10 sec trace interval (CS:80dB tone, 10 KHz, 5sec; US:footshock, 600µA, 500msec) and acquisition of fear-potentiated startle was assessed daily over a 8 day period.

Lesioned animals showed a slower acquisition of fear-potentiated startle compared to the control groups. Furthermore, the neonatally lesioned animals differed significantly from the adult lesioned animals, for they showed no acquisition of fear-potentiated startle within the 8-day period of measurement, whereas the adult lesioned animals showed a small, but significant increase of fear after 8 days of training.

These results suggest that mPFC lesions at an early developmental stage might lead to even worse deficits in a trace-conditioning task than adult lesions.

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Effects of Long-Term Isolation Housing on Behaviour in Male and Female Mice 848

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Isolation rearing of mice is one of the models to study behavioural deviations and central nervous transmission processes related to psychiatric diseases and pharmacological treatments (Rilke et al. 2001). Whereas gender specific frequencies are well known for psychiatric diseases and independent on higher disease vulnerabilities seen in women, predominantly males are investigated in animal models. On the other side in female rats specific stress responses are described and discussed to be in relation to sex specific regulatory processes of corticosterone and serotonin (Kennett et al. 1986; Haleem et al. 1988; Taylor et al. 2000). Considering our previous findings regarding central nervous serotonin regulation during isolation housing, sex dependent differences should be suggested also for our model. To reveal different adaptation processes we analysed the effects of isolation housing in a paralleled long-term study.

Male as well as female mice (NMRI, Charles River) were housed in groups or isolation for 1, 6 or 12 weeks. At the following days mice were analysed in the running wheel, open field, plus-maze, and social intruder test. Immediately after the last test mice were sacrificed by decapitation and brain regions were frozen for subsequent HPLC detection of catechol- and indolaminergic transmitters in brain homogenates. Furthermore trunk

blood was collected for corticosterone determinations. Each test was performed on one day, with 1 day of recovery between the test sessions. Running wheel activity was measured during 1 hour to determine general activity drive. The ten minutes open field test allowed the additional determination of qualitative locomotion aspects as exploration and irritation. The six minutes plus maze observation was done to analyse anxiety dependent aspects of activity. During the six minutes social intruder tests aversive and affine but also neutral, self directed behaviours were counted. Behavioural measurements were correlated with neurochemical data.

In general female mice demonstrated enhanced behavioural activities except rather reduced activities during the social intruder test. On the other hand enhanced corticosterone levels were found in females. Isolation effects on behaviour revealed different dynamics in male and female mice corresponding with different dynamics in neurochemistry and corticosterone. Results are discussed regarding interrelationships between central nervous and hormonal processes focused on dopamine, serotonin and corticosterone.

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849 Isolation-induced alterations in different AB mice strains: Autoradiographic analyses of 5-HT1A and 5-HT2A receptors

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Presented investigations were done to reveal interrelationships between exogenous triggering of and endogenous disposition to maladaptive central nervous processes. Social isolation of rodents is one of the important animal models in experimental psychiatry. Isolation housing disturbs organism-environment relationships. Coping strategies - behavioural and physiological efforts - are required (Koolhaas et al., 1999). Individuals differ in their coping strategies and capacities. Genetic background is one of the important factors. Therefore, we analysed two closely related mice strains: AB Hal and AB Gat. Studies revealed 10-20% genetic differences. They differ mainly in the expression of isolation-induced aggression. Additionally a congenic strain (CS) of the mentioned AB strains was established. At least 0.5 % genetic background derived from AB Hal, including strong vulnerability for isolation-induced aggression, was rescued in the genetic background of AB Gat (Schneider et al., 1995).

The mice were housed individually for 6 weeks. Autoradiographic analyses were done and specific binding of 5-HT1A and 5-HT2A receptors was measured in different brain regions .

AB Hal mice displayed highest specific bindings to 5-HT1A receptors in the hippocampus (CA1 field and dentate gyrus), dorsal raphe nucleus, basolateral amygdala and the cortex in comparison to AB Hal and CS. A moderate specific binding was measured in the basomedial / medial amygdala. Specific binding to 5-HT2A receptors showed lowest

values in the striatum and globus pallidus, and moderate values in the accumbens nucleus. AB Gat had lowest specific bindings in basomedial / medial amygdala, cortex and dorsal raphe nucleus, but moderate values was measured in basolateral amygdala, hypothalamus and the hippocampus (CA1 field and dentate gyrus). In contrast highest specific bindings to 5-HT2A receptors were measured in the striatum, globus pallidus, accumbens nucleus and the cortex. On the whole CS mice showed lowest 5-HT1A specific bindings with the exception of basomedial / medial amygdala. 5-HT2A receptor densities in CS mice showed similar patterns as measured for AB Hal mice.

In summary AB Hal and AB Gat mice strains differ mainly in anatomical regions functionally related to the limbic system and to systems of locomotion control. CS mice displayed a very specific pattern -similarities between CS and AB Hal could be measured in the basalganglia only.

The results were discussed considering the genetic background and our early studies examining neurochemistry and behaviour of these three mice strains (Schiller et al. 2002).

Koolhaas et al.; Neurosci Biobehav Rev 23 (7) (1999); 925-935
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Impact of homocysteine metabolites on Neuronal Network Activity detected with Microelectrode Arrays: Implications for Neurological Disturbance in Homocystinuria

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The inherited deficiency of cystathionine-β-synthase and other enzymes that are associated with methionine metabolism leads to homocystinuria – a metabolic disease characterized by mental retardation, seizures and vascular events. In homocysteinic patients levels of homocysteine (HCY), homocysteinesulfinic acid (HCSA) and homocysteic acid (HCA) are elevated in urine, serum and cerebrospinal fluid. So far it is not known which metabolites cause the disturbance of central nervous function. Here, we determined the minimal concentrations of the metabolites needed to inhibit neuronal network activity.

Hence, we investigated the dose-response curves of methionine, HCY, HCSA and HCA by recording the spontaneous spike rate of neurons growing on microelectrode arrays. Primary dissociated cortical neurons from embryonic Wistar rats, incubated in astrocytes–conditioned supplemented N2-medium, formed spontaneously active neuronal networks on microelectrode arrays within several days. During the recordings we used a head stage heating to guarantee a constant temperature of 37^0 C. After determination of the basal spontaneous spike rate (cells incubated in a buffered salt solution) the test substances were applied and the measurement started. We calculated the changes of

spontaneous spike rate (SSR) and the results were fitted by a dose-response curve given by the Hill equation resulting in an IC_{50}.

HCA and HCSA both inhibited the SSR of the neuronal network significantly at very low concentrations with an IC_{50} of 1,3 µm (HCA) and 1,9 µm (HCSA), whereas homocysteine and methionine resulted in a higher IC_{50} of 401 µm (HCY) and 412 µm (methionine). The observed effects concerning all substances were reversible after wash out. The dose-response curves of homocysteine, HCA and HCSA revealed a sharp transition, which was also observed for potent receptor acting substances like glutamate and NMDA. All three substances disrupted the burst-pattern and promoted uncorrelated single spikes similar to glutamate and NMDA.

Since HCY concentrations under pathological conditions in patients are much lower than the concentrations we determined to interfere with acute neuronal network function, the relevance of homocysteine in acute encephalopathy may be rather small. In our experiments HCA and HCSA showed strong effects on the spontaneous spike activity in concentrations about 1 µm. According to our results HCA and HCSA more likely induce the acute neurological disturbance in homocystinuria than HCY.

851 Influence of the opioid fentanyl on neuronal activity in the cat's superior colliculus

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In order to optimize anaesthesia during extracellular recording several agents have been introduced, for example halothane, isoflurane, enflurane or nitrous oxide and, in recent years, also the opioid fentanyl. However, the influence of these agents on the neuronal activity has not been analyzed in detail *in vivo*.

Fentanyl acts especially on the mu-opiate-receptor, which is densely distributed in the superior colliculus (SC). To examine the effects of fentanyl on neuronal responses of visual neurons in the SC we performed long term extracellular recording (up to 120 minutes, 120') in four chronically implanted cats under general anaesthesia (initially ketamine + xylazine, maintenance with halothane + nitrous oxide/oxygen as 3:1) and paralysis.

We analyzed the maximal stimulus driven activity as well as the spontaneous activity after intravenous boli of different fentanyl-concentrations (3, 5 or 10 mu g/kg BM). In parallel, variations of heart rate and serum-cortisol were registered as indication for experimentally induced stress.

Pharmacokinetics of fentanyl show a decrease in plasma concentration to 32% within the first hour after administration. However, our results indicate that the effect of fentanyl upon stimulus driven and spontaneous activity lasts at least 90 minutes.

Comparing the medians of neuronal activity recorded 0 – 30', 30 – 60' and 60 – 90' after administration of the fentanyl bolus with the activity occuring before the bolus, we found that the stimulus driven activity decreased up to 10 – 50% in about 38% of the

measurements. By contrast, the spontaneous activity increased by more than 150% in about 35% of the measurements. This effect was dosage dependent: an overall decrease of stimulus driven activity was found in 37% (3 mu g/kg BM), 48% (5 mu g/kg BM) and 68% (10 mu g/kg BM) of the measurements, respectively. The results concerning the spontaneous activity were more variable: an increase was found in 60% (3 mu g/kg BM), 54% (5 mu g/kg BM) and 41% (10 mu g/kg BM) of the measurements.

In conclusion, fentanyl has rather variable effects on the visual activity in the SC. Especially at higher dosages (10 mu g/kg BM) overall neuronal activity is depressed, neuronal modulation is decreased.

Dendrite formation induced by NMDA receptor stimulation: Role of the small GTPase RAC and phosphoinositide 3-kinase (П3-k) 852

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In Xenopus optical neurons, N-methyl-D-aspartate receptors activated by endogenous glutamate are involved in light induced dendrite branching (Li et al., (2002) Neuron 33:741-750). We have analyzed the mechanism of NMDA-induced dendrite formation in cultured embryonic hippocampal neurons. One day after seeding, neuronal differentiation was started by adding serum-free neurobasal medium. In cultures treated with 10µM NMDA the neurons developed within 8 h 25% more dendrites and 89 % more branches as compared to controls. Inhibition of NMDA receptors with dizocilpine (100 nM) abolished the effect of NMDA. An affinity precipitation assay was used to measure Rac and Cdc42 activity, which are known to support neurite formation. NMDA increased 3.4 fold the binding of Rac to GST-PAK-CRIB. Also Phosphoinositide 3-kinase is involved in the formation of neurites. With use of an Akt phosphorylation assay we tested whether NMDA affected the activity of П3-K signal pathway. NMDA only slightly increased 1.3 fold the phosphorylation of AKT by PDK2 at serine473. This effect was inhibited with Wortmannin (100nM). Time lapse analysis was employed to analyze how NMDA affected the addition and retraction of new dendrites and branches. Compared to controls. NMDA increased the addition of new dendrites and branches. This highly dynamic process was also influenced by Rho-kinase and П3-kinase activity. Inhibition of Rho-kinase increased the length of the dendrites and branches whereas inhibition of Phosphoinositide 3-kinase reduced the addition and retraction of new dendrites and branches. We hypothesize that NMDA induces dendrite and branch formation by activating Rac.

853 Effects of 5-HT_{2C} receptor activation on exploratory behavior and autonomic function of mice

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The serotonin receptor subtype 2C (5-HT_{2C}) has been implicated in anxiety disorders (Graeff, 1993, Rev. Neurosci. 4:181-211). Acute activation of the 5-HT_{2C} receptor by the agonists m-CPP and MK 212 induces hypolocomotion that has been interpreted to reflect an enhanced anxiety-like state (Griebel et al., 1997, Neuropharmacology 36:793-802). Therefore, we investigated the behavioral effects induced by acute activation of 5-HT_{2C} receptors by assessing exploratory behavior of male C57BL/6N mice in a new environment (novelty). The objective of the study was to evaluate whether the behavioral changes observed during novelty exposure were due to emotional activation reflected by an enhanced sympathetic activation of the autonomic system under otherwise stress-free baseline conditions in the home cage. Autonomic measures such as heart rate and mean arterial blood pressure were telemetrically recorded in the home cage of separate groups of mice after pharmacological intervention. This approach was selected to avoid any intervention with the experimental animals such as handling and novelty. Handling represents a profound stress for mice. It causes an elevation of heart rate to maximal physiological limits and invalidates any assessment of the cardiovascular system's response to emotional challenge. General arousal and conditioned fear induce tachycardia in mice (Stiedl & Spiess, 1997, Behav. Neurosci. 111:703-711).

Subcutaneous injection of the 5-HT_{2C} receptor agonists m-CPP (3 mg/kg) and MK 212 (1 mg/kg) induced hypolocomotion during novelty exposure. This hypolocomotion was blocked by pre-injection of the 5-HT_{2C}-selective antagonist SB 242084 (0.3 mg/kg) further indicating the specificity of the 5-HT_{2C} receptor involvement. This effect was also achieved by intracerebroventricular injection of m-CPP (30 μg), whereas injection of the same dose into the dorsal hippocampus elicited a mild hyperlocomotion in comparison to aCSF-injected controls. Subcutaneous injection of m-CPP (3 mg/kg) did not affect heart rate, while mean arterial blood pressure was slightly increased.

In conclusion, the lack of autonomic effects indicative of profound sympathetic activation does not support the interpretation of an anxiogenic-like effect caused by acute 5-HT_{2C} receptor activation. Thus, the induced hypolocomotion may be attributed to an inhibitory effect on the efferent motor system that may be mediated through the basal ganglia but not the dorsal hippocampus.

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In Vitro and *in vivo* regulation and mechanism of amyloid precursor protein secretion by anti-Parkinson drug rasagline

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N-Propargyl-1(R)-aminoindan (rasagiline) in now under phase III clinical trials for Parkinson's disease (PD), and it rescues dopamine neurons from cell death in animal and cellular models of PD. In the present study, we show that rasagiline (1 and 10 micro M) significantly protected rat PC12 cells against the β-amyloid ($A\beta_{1-42}$) toxicity. In addition, rasagiline significantly increased (~3 fold) the secretion of the non-amyloidogenic soluble form of the amyloid precursor protein (sAPP α) from PC12 and SH-SY5Y neuroblastoma cells. The increase of sAPP α was dose- dependent and was blocked by the hydroxamic acid-based metalloprotease inhibitor, Ro31-9790, suggesting that the effect is mediated via α -secretase activity. Using a specific inhibitor of the mitogen – activated protein kinase (MAPK) pathway inhibitor PD98059, prevented sAPP α release, suggesting the involvement of MAPK-dependent pathway in the enhancement of sAPP α release by rasagiline. Rasagiline dose dependently (0.1-100 micro M) increased the phosphorylation of p44 and p42 MAPK, which was abolished by PD98059 and by the protein kinase C inhibitor, GF109203X.

Additionally, the propargylamine, a moiety present in rasagiline, significantly increased sAPP α release, as well as MAPK phosphorylation, indicating that the propargylamine is crucial for these stimulation effects. These data suggest a novel pharmacological mechanism whereby the neuroprotective drug, rasagiline, can stimulate sAPP α secretion via activation of the MAPK pathway. In addition, rasagiline (1 micro M) induced PKC posphorylation, as well PKC translocation in PC12 cells at 1h incubation. Furthermore, Administration of rasagiline (0.1 mg/kg) to mice for 14 days significantly decreased membrane bound holoprotein APP level in the hippocampus. Consistently, rasagiline markedly increased PKC α and ε in the membrane and the cytosolic fractions of mice hippocampus. Moreover, rasagiline treatment significantly elevated the levels of myristoylated alanine-rich C kinase substrate (MARCKs), a major substrate for PKC, as well as the levels of receptors for activated C kinase 1 (RACK1). Similar effects on the holo APP and PKC levels were demonstrated for the two novel neuroprotective cholinesterase inhibitors TV3326 and TV3279 that were derived from rasagiline. These results indicate that rasagiline, TV3326 and TV3279 regulate APP processing by a PKC dependent mechanism.

In conclusion, rasagiline might prove useful to favor non-amyloidogenic APP processing, thereby reducing the formation of amyloidogenic derivatives and therefore is of potential value in Alzheimer's disease.

855 Secondary Metabolites From Marine Sponge Influence Intracellular Calcium Signals

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Despite decades of natural products isolation and structure elucidation, little is known about ecological functions of secondary metabolites from marine sponges. For example, previous studies have indicated that Caribbean reef sponges of the genus *Agelas* are chemically defended from fish predators by brominated pyrrole alkaloids (Assmann et al., 2000). Among other natural products in this series, 4,5-dibromopyrrole-2-carboxylic acid was unpalatable at natural concentrations to a common generalist reef fish, *Thalassoma bifasciatum*, in aquarium assays (Chanas et al., 1996). However, the physiological effects of bromopyrrole alkaloids to function as a feeding deterrent on a cellular basis are not yet understood. Therefore, we tested brominated pyrrole alkaloids for cell physiological effects in PC12 grown on collagen coated cover slips and in acutely isolated neurons from *Aplysia punctata*. Preliminary results indicate an interaction of 4,5-dibromopyrrole-2-carboxylic acid with cellular calcium signals. Calcium levels were measured using Fura II as a calcium indicator. When applied in high concentrations (300 µm) 4,5-dibromopyrrole-2-carboxylic acid seem to reduce depolarization (high potassium) induced calcium elevations. In general, cellular calcium plays a crucial role in many processes as in cellular signaling and secretion. One mode of antifeedant activity of bromopyrrole alkaloids against reef fish may be an interaction with the cellular calcium levels of sensory cells.

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856 $P2X_7$ receptor expression after ischemia in the cortex of rats

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$P2X_7$ receptors are ligand-gated cation channels that are activated by high concentrations of extracellular ATP. In the present study the expression of the $P2X_7$ receptor subtype in the cortex of spontaneously hypertensive rats was investigated using a per-

manent focal cerebral ischemia model. Four days after occlusion of the right middle cerebral artery (MCAO) the Western blot analysis of the cortical tissue around the area of necrosis revealed an increase in $P2X_7$ receptor protein in comparison to sham operated and untreated rats. Immunohistochemistry using antibodies raised against the intracellular C-terminal (a) and an extracellular peptide sequence (b) of the $P2X_7$ receptor subtype showed an up regulation of labelled cells in comparison to the controls. Double immunofluorescence using the two types of $P2X_7$ receptor antibodies, respectively, analysed with confocal laser scanning microscopy indicated a different localisation of the P2 receptor: on β-III-tubulin-labelled neurons (a+, b-); on glial fibrillary acidic protein (GFAP)-positive astrocytes (a+, b+); on microglial cells (a-, b-), marked with biotinylated *Griffonia simplicifolia* agglutinin isolectin B_4 (bio-GSA) or CD 11b (OX 42), respectively.

Active Caspase 3, an early marker of apoptosis, was only observed on GFAP-positive astrocytes in the periinfarct area and is co-localised with the $P2X_7$ receptor (b), too. Terminal deoxynucleotide transferase-mediated deoxyuridine triphosphate-biotin nick end labelling (TUNEL) before immunohistochemistry with the $P2X_7$ receptor antibodies confirmed the co-localisation.

In conclusion, the present data show a post-ischemic up regulation of the $P2X_7$ receptor subtype on neurons and astrocytes, but not on microglial cells *in vivo*, indicating a role of this receptor in the pathophysiology of ischemia. The co-localisation of the $P2X_7$ receptor with active Caspase 3 and TUNEL reveals its involvement in the apoptotic process.

Reduced food availability alters the expression of purinergic receptor mRNA in the nucleus accumbens of the rat

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ATP and its metabolite adenosine activate membrane receptors thought to be functionally relevant in the mediation of reward and reinforcement, e.g. feeding behavior. In a previous microdialysis study in the rat nucleus accumbens (NAc) known to be related to rewarding functions was shown that the blockade of P2 receptors inhibited the amount and time of food intake and also decreased the feeding associated dopamine release (Kittner et al. 2000). The systemic administration of an A_{2A} receptor agonist reduced the food intake in rats whereas an A_1 receptor agonist did not (Spies et al. 2002). Further, the perfusion of the NAc with a non-selective adenosine antagonist caused an increase of the neuronal power in the limbic system accompanied by an increased level of dopamine in the NAc (Krügel et al. 2003). Therefore, it is suggested that purinergic receptors may be involved in the responses to reduced food availability.

In this study the effects of restricted feeding on the purinergic transmission at the level of the adenosine A_{2A} and ADP/ATP sensitive $P2Y_1$ receptor mRNA were investigated in the rat nucleus accumbens after three days in the situation of acute food deprivation and

after ten days when adaptive changes can be expected. Furthermore, plasma leptin and the expression of its receptor mRNA (long form) to characterize the status of satiety were examined as well as the expression of the cocaine- and amphetamine-related transcript (CART) mRNA as a possible marker for changes in the sensitiveness of the reward system. Plasma levels of the anorectic peptide leptin were reduced within three days of feeding restriction; the mRNA of the leptin receptor was elevated at the tenth day. The $P2Y_1$ receptor mRNA expression showed the same time course whereas the A_{2A} receptor mRNA reached the control level on day ten after a down-regulation found on day three. CART mRNA expression was decreased in the acute situation and up regulated after ten days.

The data indicate that the accumbal gene expression during food restriction is triggered by peripheral signals like plasma leptin concentration and by neuronal signals in the CNS including extracellular adenosine and ADP/ATP and that the investigated receptor mRNAs are regulated to habituate to the reduced nutrition. In conclusion, chronic food restriction increases the mesolimbic sensitivity directed to achieve satisfaction and reward via regulation of mRNA expression involving that of $P2Y_1$ and A_{2A} receptors in a functionally antagonistic manner.

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858 Neuronal $P2X_7$ receptors in rat brain after ischemic damage

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Upon brief agonist application the $P2X_7$ receptor acts as a nonselective cationic channel by forming a large membrane pore leading to cell lysis. Only very limited knowledge exists about neuronal expression and function of $P2X_7$ receptors apart from a recently suggested influence of hippocampal $P2X_7$ receptors on gene expression. The present electrophysiological and immunohistochemical study was aimed to determine ischemia induced changes of neuronal $P2X_7$ receptor characteristics.

Untreated rat primary corticoencephalic cultures were compared with ischemically damaged cultured neurons. *In vitro*-ischemia over 5 or 30 minutes was induced by incubating the cultured cells in glucose free medium gassed with 100% argon followed by a reoxygenation period (1h-72h).

In a first step the cultures were morphologically characterised by staining with celestine blue/acid fuchsin and estimation of purine and pyrimidine nucleotide levels. A significant morphological damage as effect of ischemia was found as well as the triphosphate/diphosphate ratios were decreased.

Secondly, double immunofluorescence staining with antibodies against the $P2X_7$-receptor subtype, the neuronal marker β-III-tubuline and glial fibrillary acidic protein, a specific marker of fibrous astrocytes, was performed and analysed by using confocal laser scanning microscopy. *In vitro* ischemia resulted in a markedly increased neuronal $P2X_7$ immunoreactivity with a rather cytoplasmatic than plasmalemmal receptor-staining. Additionally, to further specify the $P2X_7$ immunoreactivity an electron microscopy study was performed using periinfarct tissue from a rat 4 days after permanent middle cerebral artery occlusion. The $P2X_7$ receptor was shown to be localised at the nuclear membrane.

Thirdly, membrane currents were recorded in the whole-cell configuration of the patch-clamp method at room temperature. In untreated cultured corticoencephalic neurons at a holding potential of -70mV no inward current responses to pressure applied ATP (100 mu M-10 mM) and the $P2X_7$ receptor preferential dibenzoyl-ATP (3-300 mu M) were found. Moreover, ischemic incubation and different duration of reoxygenation did not influence the electrophysiological unresponsiveness to either agonist.

In conclusion, the present data document an upregulation of $P2X_7$ receptor subtype of corticoencephalic cultured neurons in the cytoplasm after *in vitro*-ischemia. The negative electrophysiological results could be explained in agreement with the findings of immuncytochemistry and electron microscopy. The study documents a role of $P2X_7$ receptors in the pathological process of ischemia.

Sensitization of soluble guanylyl cyclase by YC-1 in an insect brain and its application in identifying NO targets by anti-cGMP immunohistochemistry

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Activation of soluble guanylyl cyclase (sGC) and the resultant conversion of GTP into the second messenger cGMP is a major effector pathway for the diffusible signalling molecule nitric oxide (NO). Binding of NO to the heme moiety of sGC results in a conformational change that increases the enzymatic activity by a factor of more than 100. The synthetic compound YC-1 has been identified as a potent activator of vertebrate sGC, and its precise mode of action is currently a subject of intense investigation (e.g., Bellamy & Garthwaite 2002, Br J Pharmacol 136:95).

Here we show for the first time that YC-1 is also effective in an invertebrate, the locust *Schistocerca gregaria*. Isolated brains and compound eyes (retinae without optic lobes) were incubated with YC-1 in the presence or absence of the NO-donor SNAP and/or the phosphodiesterase-inhibitor IBMX, and cyclic GMP levels were then quantified by radioimmunoassay. In parallel experiments, we investigated the cellular distribution of NO-induced cGMP in the brain by using cGMP immunohistochemistry.

On their own, YC-1 (0.05 mM) and SNAP (0.05 mM) both caused a slight increase in cGMP compared to basal levels in the locust brain. The increase seen with YC-1 on its own is presumably due to potentiation of the effect of intrinsically released NO. Co-

incubation in YC-1 and SNAP, however, resulted in a strong and highly significant ($p<0.0001$) increase in cGMP. Similar results were obtained in the retina.

A potentiation by YC-1 of NO-induced cGMP in insects is supported by our cGMP immunohistopharmacology (all experiments in the presence of 1 mM IBMX). In most regions of the brain, the number of strongly cGMP-immunoreactive neurons was low and highly variable after application of SNAP (1 mM) alone. An exception was the antennal lobe, where SNAP resulted in consistent and intense immunostaining in virtually all chemosensory afferents, and in a large proportion of interneurons. This finding conflicts with Bicker et al. (1996; Eur J Neurosci 8:2635) who reported a response in local interneurons but not in afferents.

In contrast, after exposure to YC-1 (0.1 mM) and SNAP in combination, a surprisingly high proportion of neurons throughout the brain displayed intense cGMP immunoreactivity. The signal in the olfactory afferents and interneurons was even stronger than that seen after treatment with SNAP. The widespread cGMP-response to NO revealed by YC-1 supports previous evidence that in insect ganglia, bath-application of NO-donors induces immunohistochemically detectable cGMP levels only in a fraction of the total pool of sGC-expressing cells (Ott et al. 2000; J Comp Neurol 422:521). This approach is widely used for identification of cellular NO targets in insects, and sGC may thus be much more widely expressed in the insect nervous system than previously indicated. Moreover, our findings highlight the importance of identifying the mechanisms that modulate the cGMP response to NO in insects.

860 Nitric oxide effects on the intrinsic optical signal of retinal spreading depression waves

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Spreading Depression (SD) is a wave-like phenomenon that propagates through gray matter of the central nervous system (CNS) with a velocity of 1.5-7.5 mm/min. SD is associated with several neurological disorders such as migraine aura and stroke. The main electrophysiological features of SD are a disruption of ionic homeostasis, near total depolarization of neurons and glia, transient suppression of electrical activity, and cellular swelling. Metabolic concomitances of SD are the reduced level of energy-rich molecules (glucose, ATP and phosphocreatin), and increased level of mitochondrial oxidation and lactate.

The propagation of SD waves is associated with a change of the intrinsic optical signal (IOS) of the tissue. The strongest change of IOS was observed in the chicken retina. The retinal IOS (rIOS) of SD has two components: an early short lasting and a late long lasting phase. The first peak correlates in time and duration with the electrophysiological concomitants. The second peak is mainly influenced by metabolic factors, but precise information about processes underlying this phase is still missing.

Here we show that nitric oxide (NO) changes the parameters of the late phase of the SD optical signal. For this aim we have tested the effects of some NO-donor substances (nitroprusside and trinitroglycerine) on the whole IOS. The influence of potassium-hexacyanoferrat and potassium cyanide (control for nitroprusside) and glycerine (control for trinitroglycerine) were investigated, too. Among the investigated parameters of the IOS only the duration of the late peak of SD waves was not influenced by nitric oxide. The effect of potassium cyanide (KCN) on IOS is interesting not only because this is a control substance for nitroprusside, but also because KCN is a specific blocker of cytochrome oxidase and consequently is a blocker of mitochondrial respiration. KCN significantly prolongs the duration of the earlier phase and the magnitude of the late phase of the SD optical profile.

The modulation of Methylphenidate-induced motor activity in rats by melatonin and vasopressin 861

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The psychostimulant Methylphenidate (MPH), while commonly prescribed for the treatment of attention-deficit hyperactivity disorder, is well known to enhance motor activity in a dose dependent manner and in part depending on the time of its application during the light-dark cycle. The neurochemical mechanisms have been attributed to an increase in the dopamine (DA) concentration by inhibition of the synaptic DA reuptake. Moreover, after MPH administration the hypothalamo-neurohypophysial axis including the neuropeptide vasopressin (AVP) was found influenced. Both the latter and behavioural effects of central AVP can be modulated too by the pineal gland with its light-dark dependent activity. Therefore the present study was performed to investigate whether the pineal gland, its hormone melatonin (Mel), and AVP are involved in the MPH-evoked stimulation of activity.

After s.c. application of 10 mg/kg MPH the motor activity in pinealectomised (PE) rats was significantly higher than in sham-operated (SO) animals. After application of 250 µg Mel before MPH treatment the stimulation of motor activity was diminished in PE rats and augmented in SO animals; however, when SO and PE rats were compared after Mel pretreatment the reaction to MPH was near identical. Blocking the endogenous AVP by 25 µg or 1 µg of the AVP-V1a receptor antagonist d(CH2)5[Tyr(Me)2]AVP before MPH treatment significantly augmented the motor activity in SO rats only and abolished the differences seen between SO and PE animals after MPH application. The present results indicate that the behavioural stimulation of MPH were modulated by both the pineal gland with its hormone Mel as well as the neuropeptide AVP. Considering the fact that the effects of MPH has been mediated through biogenic amines and the metabolism of these transmitters can also be influenced by both Mel and AVP, then such functional relation would seem to provide an explanation for different behavioural response in PE and SO rats, and may explicate complex possibilities of behavioural modulation, too.

862 Effects of Arsenicals on Neuronal Ion Channels

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Methylation to tri- and pentavalent organoarsenicals is considered to be a main detoxification pathway for inorganic arsenicals. Pentavalent monomethylarsonic acid ($MeAsO(OH)_2$; MMA^V) and dimethylarsinic acid ($Me_2AsO(OH)$; DMA^V) belong to the major metabolites of inorganic arsenicals detected in urine. Trivalent compounds such as methylarsonous acid ($MeAs(OH)_2$; MMA^{III}), which are considered as intermediates in enzymatic detoxification, have also been identified in urine samples. Recently, the question of whether the generally accepted metabolism of inorganic arsenicals really results in detoxification or whether these methylated species also have deleterious biological effects was raised (e.g. Styblo et al. 2000; Petrick et al. 2000; Vega et al. 2001).

In this study, the effects of trivalent arsenous acid (H_3AsO_3; iA^{III}), the trivalent and pentavalent organic arsenic compounds MMA^{III} and MMA^V and the pentavalent DMA^V were tested on neuronal voltage- and ligand-operated ion channels from rat brain heterologously expressed in Xenopus oocytes. Membrane currents of ion channels were measured by conventional two-electrode voltage-clamp techniques. Arsenous acid and the pentavalent organic arsenic compounds were tested in concentrations of 0.1 to 100 µmol/l, the trivalent organic compound in concentrations of 0.001 to 10 µmol/l.

Voltage-operated ion channels: The inorganic and organic arsenicals had neither effects on potassium channels (Kv1.1, Kv1.2, Kv2.1, Kv3.1) nor on sodium channels (typeII).

Ligand-operated ion channels: The principal finding of this study is that the inorganic iA^{III} had no significant effects on glutamate receptors of the NMDA-, AMPA- and the mGluR-type, whereas the organic arsenicals MMA^V and DMA^V affected the ionotropic glutamate receptors in a different and concentration-dependent manner: MMA^V significantly enhanced NMDA-mediated response with a threshold concentration of <10 µmol/l, whereas the AMPA receptor-mediated responses were unaffected by this substance. DMA^V significantly reduces both the ion currents through AMPA- and NMDA-receptors with threshold concentrations of <1 µmol/l. Responses mediated by the metabotrope glutamate receptors were not affected by any of the tested compounds. Investigations with the analogous trivalent compound MMA^{III} are currently in progress and point to a reduction of the AMPA receptor-mediated and to an enhancement of the NMDA receptor-mediated responses.

In summary, the oocytes can be used as a tool for neurotoxicological investigations, since any change in ion channel function may be taken as proof of the neurotoxic potency of the tested substance. The effects of MMA^V and DMA^V on glutamate receptors point to a neurotoxic potential of these organic substances. Compared to the (absent) effect of inorganic iA^{III} on these ion channels, the organic arsenicals possess a considerably higher neurotoxic potential. (Supported by Deutsche Forschungsgemeinschaft)

Modulation of cortical excitability by atypical neuroleptics 863

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Objectives Using transcranial magnetic stimulation as a diagnostic tool, cortical excitability was studied in healthy volunteers under the influence of two atypical neuroleptics, clozapine and amisulpiride. Methods Motor threshold, cortical silent period, intracortical inhibition and facilitation were studied in fifteen healty volunteers before and after ingestion of clozapine or amisulpiride. Results The principal finding was a different excitatory profile between both drugs, reflecting structural differences and differences in receptor-binding profiles and pointing to a fundamental role of the dopamine D2 as well as the dopamine D4 receptor as modulators of cortical excitability. Conclusions TMS sees to have the potential to detect neurophsiological differences within the class of atypical neuroleptis, which may be helpful for clinal decisions in teating patients.

Neuromodulatory effects of SSRIs on cortical excitability influenced by genetic factors 864

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Objectives Antidepressant efficacy of selective serotonin reuptake inhibitors (SSRIs) have been shown to depend on functional polymorphisms within the promoter region of the serotonin transporter gene (5-HTTLPR). This gene gives rise to a biallelic polymorphism designated long (l) and short (s). Homozygosity for the long variant (ll-genotype) is associated with a two times more efficient 5-HT uptake compared to the s/l- or s/s-genotype. Paired pulse transcranial magnetic stimulation is a feasible tool in detecting changes of motor cortex excitability induced by psychopharmacological agents. This pilot study aimed to measure neuromodulatory effects of SSRIs on cortical excitability in healthy volunteers characterized by distinct genotypes of the 5-HTTLPR. Methods Cortical excitability was determined in eight genetically defined subjects pre and post ingestion of 60 mg citalopram. Results Subjects with the ll-genotype of the 5-HTTLPR showed a significant enhancement of intracortical inhibition as compared to volunteers without the ll-genotype. Conclusion Distinct neuromodulatory effects after intake of citalopram basing on allelic variations of the 5-HTTLPR may explain variable response of patients treated with SSRIs.

865 Electrical neuronal network activity on a silicon based neurosensor chip with flow injection system

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For more than twenty years Multi Electrode Arrays (MEAs) are used to record the electrical activity of cultured primary neuronal networks. In our current project we want to establish a silicon based sensor chip for measuring of electrical activity with metallic electrodes and Cell Potential Field Effect Transistors (CPFETs).

Primary neurons were taken from the spinal cord or the frontal cortex of fetal mice *(Mus musculus)*. In experiments we could show the feasibility of silicon neurosensor chips for measuring of electrical neuronal network activity in a chamber with 7 mm diameter and 200 µl volume. Good measurements of activity were executed with metallic electrodes.

Ion Sensitive Field Effect Transistors (ISFETs) are used to measure changes of the pH-value caused by metabolic activity. Measurements of acidification of neuronal networks have been succesfully executed on ISFET-chips.

The new generation of this chip contains ISFETs to measure acidification as well as electrodes and CPFETs to measure electrical neuronal network activity. The measurement of acidification requires a small measuring chamber with 10 µl volume as well as the periodic exchange of the medium to prevent toxic effects of decreasing pH-value. For this purpose we have realized a flow injection system which allows the periodic exchange of fluid in the recording chamber. We have tested the influence of the fluid injection system on the neuronal activity.

In most cases the electrical activity remains alive after mounting of the flow injection system. We could measure an increase of electrical activity during pumping phases accompanied with a total loss of bursting regularity. In resting phases the electrical activity was lower respectively to measurements without flow injection system.

At present we minimize the negative effects of the pumping activity by reducing the pumping rate and the use of a semipermeable diaphragma between the recording chamber and the pumped fluid. In addition we want to test the feasibility of the CPFETs for measuring electrical neuronal network activity.

866 Impact of spike sorting noise on features describing spike and burst activity of neuronal networks on MEAs

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We studied the robustness of features under noisy conditions. Noise in this context of neuronal activity (spiking) is not considered the analogue noise at the electrodes, but

spikes which were falsely detected or falsely missed by the spike detection algorithm as a result of imperfect spike sorting. Hence this noise is termed 'spike sorting noise'. This noise is at present unavoidable and will remain even with automated algorithms, because there is always a trade-off between the number of recorded neurons (units) and the quality of the recorded spike trains.

Matured neuronal cultures from murine frontal cortex tissue grown on microelectrode arrays (CNNS, Denton, Texas) were the base of the analysed experiments. To quantify the impact of spike sorting, spike trains of recorded activity were analysed. The recorded spike trains exhibited no apparent abnormal activity, e.g. increased interburst spiking or divergent burst patterns, and thus would be included in normal analysis.

Data analysis was performed using NeuroExplorer (Dallas, Texas) yielding data for 19 features: 11 means, 8 accompanied by their variation across time ('CVtime', bin size 60 seconds). After the initial analysis, the spike trains were re-sorted off-line using OfflineSorter (Plexon Inc., Texas), and the percentage of false positive and missed spikes were calculated. Subsequently, the spike trains were analysed again and the results compared with the previous values.

When comparing spike trains (n=30) before and after re-separation, false positive (4.1±4.3%) and missed (2.8±4.6%) spikes were found. The features affected most were spike rate, since it is directly linked to the number of spikes, but also CVtime of mean frequency in burst as well as CVtime of peak frequency in bursts.

One way of interpreting data is to normalise native activity, i.e. setting its features' values to 100%, and use the relative change of the features after stimulation for statistics. Originally recorded (noisy) and then de-noised activity after application of various substances were compared to their respective native activity (n=19). In these cases, three features showed significant deviations ($p<0.05$), but the average over all features showed no significant deviations (98.8±5.6% of original values) when comparing noisy and de-noised spike trains.

In conclusion: if you sort carefully, you can trust your data.

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Neuroprotection and neuronal dysfunction upon repetitive inhibition of oxidative phosphorylation 867

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Repetitive inhibition of oxidative phosphorylation is an established model of neurodegeneration. In contrast, a single mild treatment can be neuroprotective – chemical preconditioning. Repetitive chemical inhibition of oxidative phosphorylation may thus be a tool to study deterioration and improvement of cellular hypoxic tolerance and subsequent differential regulation of cellular responses in the same model.

We investigated murine hippocampal function upon repetitive intraperitoneal injections of 3-nitropropionate (3-NP; 20 mg/kg body weight), an inhibitor of mitochondrial complex II.

Expression of mRNA for adenosine-A1- and –A3-receptors and endothelial and neuronal nitric oxide synthase remained at control level for both treatment intervals. In contrast, depending on the time interval of treatments we found distinct regulation of nerve growth factors (NGF, BDNF) and synaptic functions (as assessed with population spikes and exploration of radial mazes).

868 Anterograde transport of GFP-tagged neurofilaments in living cells

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The purpose of the present study was to investigate the correlation between anterograde transport of neurofilaments (NF) and the cytoskeleton in neuronal cells. Transport mechanism evoke the physiological function of neuronal cells, as macromolecules and cell organelles have to be transported to their sites of utilization, after synthesis in the cell somata. Traditionally axonal transport is distinguished into slow and fast anterograde transport, referring to the transport velocity. Recently it was detected that the average rate of transport reflects a combination of fast movements and pauses (“stop and go traffic”; Brown, 2000)

A suitable method to study transport processes in neuronal cells under varying conditions is the direct injection of macromolecules into the cell somata (Meller, 1994). Therefore we used cell cultures of dissociated chicken dorsal root ganglia as well as Purkinje cells in rat cerebellar slice cultures, to inject a vector containing a NF-M-eGFP reporter transgene into individual cells. Analysis of NF-M-GFP transport was performed by means of “fluorescence recovery after photobleaching” (FRAP) with a confocal laser scanning microscope (Zeiss, LSM 510 Meta).

Unlike unconjugated GFP 24h after microinjection of the vector into single neurons, NF-M-GFP was excluded from the nucleus, whereas NF-M-GFP filaments were clearly visible in the somata and also transported over long distances into the DRG neurites (reaching several millimetres). Time-lapse imaging at short intervals revealed rapid movements of fluorescent dots, representing GFP-tagged NF-subunits, through bleached regions. FRAP was blocked by treatment with the microtubules depolymerising drug colchicine.

Therefore, the presented data implicate that (1) neurofilaments are transported along microtubules and (2) neurofilaments are transported at small subunits, probably oligomers in dissociated DRG and Purkinje cells of rat cerebellar slice cultures.

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The role of NCAM phosphorylation on NCAM mediated signal transduction pathways 869

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The neural cell adhesion molecule (NCAM) is a posttranslational modified protein with multiple phosphorylation sites located in the cytoplasmic tail. To study the role of intracellular phosphorylation of human NCAM, one potentially phosphorylated amino acid was exchanged against an amino acid which can not be phosphorylated. The neuronal rat B 35 cell line which shows low endogenous NCAM expression was stably transfected with wild type and mutant human NCAM 140 and NCAM 180 cDNA, respectively. Positive clones with nearly identical expression levels were chosen for further analysis. We compared the phosphorylation levels of wild type and mutant proteins and measured the effects of the mutation on different signal transduction pathways and neurite outgrowth.

In vitro differentiation of neural progenitor cells on a *biofoil* membrane system 870

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Although the effect of oxidative stress is not completely known, it has been hypothesized that it participates in the degeneration process of certain neuronal populations in Parkinson's disease. The principal disorder in Parkinson's disease is the massive loss of dopaminergic neurons of the substantia nigra.

Previous studies exemplified the importance of the investigation of proliferation and consecutive differentiation of neuronal precursors under the influence of oxygen as a way to understand the mechanism of this nerve cell death.

In the present study, we observed the influence of matrices, culture medium compounds and oxygen partial pressure on the generation of dopaminergic neurons in cultured rat mesencephalic precursors. In addition to these experiments, we examined the effects of various concentrations of the antioxidant, ascorbic acid. To determine the effect of oxidative stress, we used a produced membrane system, whereby the oxygen is able to permeate the tissue from both sides (above/below). For comparison, ventral mesencephalon cells were differentiated both in conventional multiwell plates and on a *bioFoil* membrane system. The characterization of cultured cells was performed both by immunocytochemistry for several neuronal cell types and by high performance liquid chromatography in order to analyze specific neurotransmitter concentrations. Furthermore, we observed the relative expression of total cellular RNA by using PCR amplification with different gene-specific primers. Besides this observation, a gene expression analysis was performed by the establishment of an neuronal chip system using the DNA *microarray* technique.

Our experiments resulted in a significant increase of proliferation after seven days in culture due to various culture conditions. Additionally, we found that approximately 40% of the expanded precursor cells can be induced to express tyrosine hydroxylase. However, a significant decrease of the yield of tyrosine hydroxylase positive cells could be demonstrated after cultivating the cells at a higher oxygen tension.

These results suggest that the *bioFoil* membrane could increase the vulnerability of neuronal cells to oxidative stress, and indicate that the choice of culture conditions strongly influences the differentiation of dopaminergic neurons. Also, variances in the differentiation activities of mesencephalic precursors to dopaminergic neurons demonstrate a significant influence of oxygen tension in conjunction with the culture matrix. We conclude that the used membrane system could be a feasible way to analyze tissue cultures with regard to the oxidative conditions.

871 Neurophysiological characterization of cryopreserved rat cortical neurons on microelectrode arrays

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Neuronal networks growing on microelectrode arrays (MEA) are promising tools for assessing network dysfunction *in vitro*. Network responses to pharmacological agents have already been investigated in cultures of murine spinal cord and cortical neurons as well as on rat cortical and hippocampal neurons. Recently, cryopreserved embryonic rat cortical neurons have become commercially available (QBM Cell Science, Canada). We characterized this cell culture with respect to morphological markers and electrophysiological network properties and further examined their applicability as a cell based biosensor for detection of neuroactive substances in human cerebrospinal fluid.

Cell suspensions of thawed cortical neurons from Sprague-Dawley rats (embryonic day 18) were plated on MEA with a square grid of 60 electrodes (30 µm diameter, 200 µm spacing; Multi Channel Systems, Germany) at a density of 2 x 10^5/cm^2. For each substance examined, the IC_{50} was determined from a dose-response curve based on the change of spontaneous spike rate. Staining of neuron-specific markers (neurofilament, MAP-2, synaptophysin), astrocytes (GFAP) and DNA (DAPI) was performed on coverslips for immunocytochemical characterization.

Results:

1. Double-labeling experiments with GFAP and neurofilament revealed a neuron-astrocyte-ratio of approximately 50/50. Neurons further expressed MAP-2 and synaptophysin.

2. Cryopreserved neurons formed a neuritic network. Spontaneous spike activity and periodic burst patterns could be recorded repetitively from 11 to 84 days in culture similar to acutely prepared neurons.

3. Dose response curves revealed the following electrophysiological properties: TTX, GABA and APV inhibited the spontaneous spike activity by prolonging the interburst intervals in a dose-dependent manner with an IC_{50} of 1.1 nM, 1 µm and 18 µm, respecti-

vely. NMDA and glutamate responses were biphasic, inhibitory at higher concentrations (IC_{50}=1.8 μm and 10 μm), but excitatory below their IC_{50}. 4. Application of cerebrospinal fluid (CSF) from patients with benign intracranial hypertension led to a two-fold increase in network activity compared to artificial CSF. Thus, human CSF seems to contain network activating substances.

Conclusion: Cryopreserved cortical cells on MEA developed electrophysiologically active neuronal networks with stable spike and burst activity. The IC_{50} values demonstrated a higher sensitivity to neuroactive substances than published results of devices using murine spinal cord neurons. We conclude that cryopreserved neurons on MEA are promising tools for detection of clinically relevant neuroactive substances even in the CSF of patients.

Intracellular calcium signalling in a motoneurone cell line and non motoneurone cell line - implications in study of amyotrophic lateral sclerosis. 872

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Motoneurones are selectively damaged in human Amyotrophic Lateral Sclerosis (ALS) and corresponding mouse models of ALS, a process that has been linked to their exceptional vulnerability to calcium-mediated excitotoxic events and associated cellular disturbances. Previous studies from our group has indicated both an exceptionally low concentration of calcium buffers and a particularly prominent role of mitochondria in regulating [Ca++]i, contribute to selective vulnerability of motoneurones.

In this report we investigated the motoneurone cell line, NSC-34, for the role of internal organelles in regulating [Ca++]i. NSC-34 cell line, transfected with mutant human SOD1 has been previously established as an attractive model system to study ALS related molecular cascades. The role of mitochondria in the regulation of [Ca++]i was investigated by bath application of FCCP, a mitochondrial uncoupler that collapses the mitochondrial membrane potential. FCCP application shows a dramatic rise in the [Ca++]i indicating a massive release of Ca++ from mitochondrial-related stores. Similarly, bath application of the SERCA pump inhibitor thapsigargin (TG) was accompanied by Ca++ release of comparable amplitude.

These data were compared with identical measurements performed on a non-motoneurone cell line SH-SY5Y (human neuroblastoma), which showed substantially smaller FCCP and Thapsigargin induced responses in [Ca++]i. Taken together, our data on cell culture models, i) underline the important role of organelles in regulating [Ca++]i in motoneurones and ii) identify NSC-34 as model system to study the molecular basis of selective vulnerability and ALS-related signal cascades.

873 Recombinant Semliki Forest virus effectively transduces primary neuron cultures, but vector toxicity limits its use *in vitro*

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Transduction of primary neuronal cultures by viral vectors is a powerful tool for structural and functional gene analysis. Semliki Forest Virus (SFV) has previously proved to be a vector system which effectively infects cell lines and organotypic slice cultures and can be rapidly generated under S1 safety conditions. The aim of this study was to evaluate the suitability of SFV vectors for the transduction of primary midbrain and hippocampal neuron cultures and to employ the vector system in functional assays.

In order to study transduction efficiencies, time and temperature kinetics as well as cell type specificity of expression we produced SFV vectors encoding for Enhanced Green Fluorescent Protein (EGFP). Vectors encoding for anti-apoptotic proteins Bcl-X_L and XIAP were designed in order to test their applicability in functional assays of neuronal apoptosis.

Serum free primary midbrain cultures showed a rapid expression of recombinant protein as early as 4-6h after infection. Transduction efficiencies could be increased by 15-20fold when cells were kept at 31°C instead of the usual 37°C reaching nearly total transduction of the culture. Proportional transduction was seen in the dopaminergic subpopulation, which accounts for about 10% of the midbrain culture. However, SFV vectors showed a marked cytotoxicity for cultured neurons which was clearly visible about 48-72h after infection. Transduced cells showed sequestrations of cytoplasm containing recombinant EGFP indicating a massive overproduction of the *per se* non-toxic protein. Similar results were obtained with primary hippocampal cultures.

Glutamate toxicity for hippocampal neurons and MPP^+ intoxication for midbrain dopaminergic neurons were used as paradigms for neuronal apoptosis. Experimental setups were limited to 48h after infection in order to minimize cytotoxic effects. However, overexpression of anti-apoptotic proteins Bcl-X_L and XIAP by SFV failed to increase cell survival in both paradigms. Even within a 48h time range recombinant protein was rapidly sequestered by infected cells accounting for a dose dependent vector toxicity.

We therefore conclude that SFV vectors can be used for effective transduction of primary neuron cultures in short term paradigms addressing structural questions. The use of this vector system for functional *in vitro* studies, however, is limited by vector-inherent cytotoxicity due to overproduction of recombinant protein.

Rapid differentiation of NT-2 human teratoma cells into postmitotic neurons 874

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Cells from the human teratocarcinoma cell line NT-2 can be differentiated into postmitotic neurons when subjected to low concentrations of retinoic acid (Pleasure et al, Journal of Neurosience 12(5): 1802-1815, 1992). This differentiation process takes between 42 and 54 days. Here, we propose a modified differentiation protocol which reduces the time needed for differentiation considerably without compromising the qualtity of the neurons obtained. The introduction of an additional cell – aggregation step, cuts the total time needed to obtain high yields of highly purified NT-2 Neurons to about 24 – 28 days. The cells obtained showed neuronal morphology and migrated to form ganglion – like cellular clusters. These cell clusters were linked to each other by bundles of neurites. Differentiated cells were imunostained for the cytoskeleton associated proteins MAP2 and Tau. Moreover the cell bodies were imunoreactive to the ELAV – like neuronal RNA – binding protein HuC/D which has been implicated with neuronal develpoment and differentiation.

The rapid production of NT-2 derived terminally differentiated nerve cells will facilitate further studies on the molecular processes during human neuronal differentiation. This cell culture system is also useful for replacing animal experiments aimed at screening neuroprotective drugs.

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Investigations on kinetics of cell-transistor coupling by means of genetically modified HEK293 cells 875

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The electrical coupling of neuronal cells to metal microelectrodes and non-metallized field-effect transistor (FET) gates has been the subject of intense studies during the recent years. There are many different applications where the extracellular non-invasive signal recording offers a new, very promising characterization tool, especially in pharmacological and neurological studies.

For the investigation of the extracellular coupling of individual cells to FETs, patch-clamp experiments with genetically modified HEK293-cells cultured on the silicon oxide-layer of the transistor surface were performed. The cells are stably transfected with the K^+-selective ion channel b-EAG1, which gating times can be varied in voltage-clamp mode. Differences in holding potential and different duration of pre-pulses to the

stimulation pulse as well as differences in bivalent cation concentration (e.g. Sr^{2+}, Ba^{2+}, Zn^{2+}, Mg^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}) in the extracellular medium are changing the gating times of the K^+-efflux in a wide range (several ms to s).

The response of a cell that adhered directly on a transistor-gate is monitored by patch-clamp recordings and a FET simultaneously. In this way the influence of the K^+-ionic currents to the resulting extracellular recordings can be dynamically investigated. The amplitude of the FET-signal strongly depends on the seal between cell and transistor. In these measurements the FET overestimates the potassium signal as previously described.

We explain the extracellular recorded signal shapes using an equivalent electrical circuit, the Point-Contact Model, implemented in a standard simulation program for electrical circuits called Spice. It has been reported that the high response amplitudes of potassium currents in the transistor signal indicate an accumulation of specific K^+-type potassium channels in the contact region. We think that the higher signal amplitude and the channel-dynamics cannot simply be explained only by accumulation of channels in the cell-transistor contact, and therefore investigate other possibilities like e.g. electrochemical effects.

876 Rabbit retinal organ culture as an in-vitro-model for hepatic retinopathy

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Hepatic failure (HF) induces morphological and pathophysiological changes in the brain (these are collectively termed as hepatic encephalopathy [HE]), but also in the retina. Morphological and/or electrophysiological characteristics of hepatic retinopathy (HR) have been reported in humans suffering from HF, in an animal (TAA) model of acute liver failure, and in Müller cell cultures grown at high concentrations of ammonia, a major pathogenic factor in HE or HR. Here we aimed at establishing and characterizing an easily accessible in-vitro-model for HR.

Retinal pieces of newborn rabbits were cultured under standard conditions (MEM supplemented with 10% FCS; 37°C; 5%CO_2) up to 22 days *in vitro* (DIV). After one week in culture, ammonia was added to the medium (NH_4Cl at f.c. of 0.5, 1.0, 3.0, and 7.0 mM; up to day 22). The cultures were either routinely processed for light and electron microscopy, or subjected to immunocytochemical reaction with antibodies against glia-specific marker proteins (GFAP, GS, Vim) and the protoncogene protein Bcl-2.

In general, the structure of the retinal explants was still well preserved after 22DIV. We found only few rosette-like structures with intact layering of retinal cells, as well as some apoptotic figures and Müller cells disrupting the outer limiting membrane (OLM), sparsely distributed throughout the control explants after long-term culture.

In accordance with previous results obtained with developing rabbit retinas *in vivo* we found the expression of Bcl-2 at DIV8 limited to the Müller cell endfeet; only a faint staining of their proximal processes was observed in the inner (IPL) and inner nuclear

layers (INL). At DIV15 we observed strong staining of the Müller cells throughout all retinal layers. In long term cultures, Bcl-2 expression is also visible in subretinal structures, showing that some of the Müller cells move from their original position in the retina into the subretinal space. There were no pronounced changes in Bcl-2 expression in cultures grown at different concentrations of ammonia, although increasing structural alterations were caused by higher ammonia concentrations (e.g., thickening of the Müller cell processes, and irregular appearance and position of MCs). Only when cultured with 7.0 mM NH_4Cl, the MC staining looks patchy, reflecting severe damage and onset of cell death.

At the ultrastructural level, a stepwise disintegration of the tissue cultured under hyperammonemic conditions was noted, eventually manifested as a loss of both plexiform layers. MCs showed features typical for hepatic retinopathy and symptoms of increased metabolism, such as an accumulation of mitochondria, vacuoles filled with neuronal debris, and, finally, apoptotic figures. The distal MC processes (adjacent to the OLM, where the vast majority of mitochondria are situated) appeared swollen. In summary, the established culture model seems to be a promising tool for detailed *in vitro* studies of the pathomechanism of HR.

Control of Attachment and Growth of Rat Hippocampal Neurons in Culture **877**

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Typical culture methods favor formation of neuronal cytoarchitecture in patterns that are often too complex to delineate. Simpler networks are desirable for studying functioning, plasticity and development of nerve cells on a more fundamental level, in a better controlled environment. The key to such systems is the control of attachment and growth of neurons. Here we present a modification of the technique based on contact guidance that has been utilized previously in studies on snail neurons.

Artificial tissue culture substrata consisted of glass coverslips or oxidized silicon wafers covered with a biocompatible thick photoresist layer (SU-8) patterned on the micrometer scale (grooves with Y-shaped intersections). The substrata were uniformly coated with poly-D-lysine and laminin-1. Neurons from E18 Wistar rat embryo hippocampi were plated in serum-free medium, positioned and cultured for over two weeks.

Our technique provides sufficient mechanical support for cell bodies and we were able to build networks of two to three neurons with functional synaptic connections, as can be shown by immunocytochemical localization of synaptophysin, AMPA-selective glutamate receptors and also by FM1-43 staining of active presynaptic terminals after K^+ depolarization. In contrast to cell bodies most neurites were not affected by topographical cues.

The presented technique provides a dependable tool to immobilize small neuronal cell bodies on predefined positions and to build simple defined neuronal networks. This is an important step towards our aim of reliable electrical interfacing of individual nerve cells

and semiconductor microstructures, as it prevents the somata from migrating away from the recording/stimulating sites during neuronal growth.

878 Cryo-electron tomography of cultivated mammalian neurons

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We use a novel method of cryo-electron tomography to investigate the morphology of neuronal cells preserved by rapid freezing. Our method utilizes the resolving power of the electron microscope and provides a true three-dimensional view of the structures as they occur *in vivo*. Recent applications demonstrate that the method is particularly useful for very large and complex samples, thus opening up a completely new way for visualising cells at molecular resolution (1).

We decided to study the morphology and structural details of specialized domains of neurons cultivated directly on electron microscopy grids. Our long-term goal is to depict the intriguing structural details and organization of cytoplasmic elements within dendrites, axons, spines and synapses at molecular resolution.

At the moment we focus our efforts to progress in three major technical advancements: cultivating healthy cell cultures on the EM-grids, recording high quality tomographic data as well as on computational data analysis.

1. The sample: Hippocampal neurons isolated from rat and mouse embryos were planted on Au or Ni electron microscopy (EM) grids coated with formvar, carbon and poly-L-lysin (2). After at least 2 weeks in culture, the cells were preserved by rapid freezing in the native, hydrated state.

2. Microscopy: Frozen samples were imaged at cryo conditions in the Philips CM300 electron microscope with the minimum electron doses to produce tilt series of some 60-100 images that yield three-dimensional tomographic reconstructions. The critical issue here is to compromise between electron doses that must be high enough to generate necessary signal and at the same time not exceed the critical value that would destroy the electron beam sensitive sample.

3. Image processing: Applied in order to recognise the structural details of interest in the crowded environment of intact cytoplasm.

Our reconstructions enable us to visualise for the first time an intact cytoplasmic organisation of neuronal dendrites and axons. At the resolution of 4-6 nm the cytoskeletal elements (microtubules, neurofilaments) are particularly clearly depicted. We focus on the organization of individual microtubules as well as their bundles and pay special attention to the associated molecular and vesicular features as potential objects to address the issue of intracellular transport. Recently also actin filaments were visualised

in the tomograms. Similarly the vesicles in cytoplasm often follow certain defined patterns of sizes and distribution indicating their origin as synaptic, endocytic or transport vesicles. In addition, the organelles and molecular assemblies such as mitochondria, ER, polysomes and ribosomes are currently being analysed in more detail. We intend to increase the fidelity in specific recognition of morphological and molecular features by applying *in vivo* the electron dense markers directed highly specifically to the sites of interest.

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Topologically Defined Networks of Mollusc Neurons Electrically Interfaced to Silicon Chips **879**

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A detailed investigation of neuronal networks requires a defined topology of the synaptic connections and a stimulation and recording technique that allows long-term supervision of the neurons involved. In previous studies, field-effect transistors and capacitive stimulation areas on silicon chips were shown to be suited for non-invasive electrical interfacing of mollusc neurons [1]. Recently, we obtained small networks of defined geometry using topographical guidance of snail neurons [2]. Here we present a combination of these two methods.

We fabricate silicon chips with arrays of two-way contacts of capacitive stimulators and field-effect transistors. On the surface we process topographic polyester structures consisting of pits that are aligned with the two-way contacts and of narrow connecting grooves. Neuronal somata are dissociated from Lymnaea stagnalis and placed into the pits. The grooves guide the outgrowth of neurites and hold them in their grown geometry with the cell bodies being immobilized by the pits. Electrical synapses are formed when the growing neurites encounter in the grooves. Individual neurons of small nets are capacitively stimulated by voltage pulses applied to the chip. Signals pass through synapses and trigger action potentials in postsynaptic neurons where the source drain current in the transistors underneath is modulated.

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880 Heat Shock Proteins in Cultured Rat Brain Neurons: Developmental Expression and Differential Regulation after Stress

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Heat shock proteins (HSPs) play a role in cell differentiation and apoptosis. HSPs act as molecular chaperones and can be upregulated in response to various kinds of stresses. The stress response may play a protective role and provide tolerance against further stress situations. However, HSPs do not only have beneficial effects. Increased levels of stress proteins are often found under pathological conditions, e.g. as a constituent of intraneuronal inclusions in Alzheimer's disease.

Here the developmental expression of HSPs in primary neuronal cultures, derived from the brains of embryonic rats, was investigated. Additionally, the differential response to stress situations, e.g. heat stress (44°C, 30 min) and oxidative stress (H_2O_2; 100 µm, 30 min), was tested and compared to astrocytes. Cellular extracts were analysed by immunoblot procedure using a variety of antibodies against different HSPs. GRP94 and 78 are members of the glucose-regulated proteins, induced by perturbation of the endoplasmatic reticulum. HSP90 is a cytoplasmatic chaperone interacting with the cytoskeleton. HSC70 and its inducible counterpart HSP70 cooperate with HSP90 in the degradation of misfolded proteins. Members of the HSP60 family participate in the folding and assembly of transported proteins into the mitochondrion. HSP40 interacts with HSP70 to promote protein folding and repair misfolded proteins. HSP32 (heme oxygenase 1) has been suggested as a sensor of oxidative stress. The small HSPs, HSP25 and alphaB-crystallin, are involved in cytoskeletal organization and are specifically found under neuropathological conditions.

The data show that in neurons, cultured up to four weeks, HSP90, HSC70, HSP60, HSP40, GRP94 and 78 are constitutively expressed at all times of *in vitro* differentiation, while HSP70 and alphaB-crystallin are not detectable. HSP32 and HSP25 are present in very low amounts. In astrocytes HSP70 is not detectable, while HSP25, alphaB-crystallin and HSP32 are constitutively expressed. When neuronal cultures (7div) were subjected to heat stress (HS), the induction of HSP70, HSP32, HSP25 and alphaB-crystallin was observable, while HSP90, HSP60, HSP40, GRP94 and 78 remained unchanged. After oxidative stress (OS), only HSP32 was induced in neurons. In astrocytes, the induction of HSP70 and HSP40 was observed only after HS, while the upregulation of HSP32 and alphaB-crystallin was detectable after HS and OS. Hence, HSP70 is upregulated in neurons and astrocytes after HS only, HSP32 after HS and OS. alphaB-crystallin is constitutively expressed in astrocytes and inducible after both stress situations, but can be detected in neurons only after HS.

In conclusion, the constitutive and inducible expression of HSPs is different in neurons and glia, indicating cell type specific roles and their functional significance during development and establishment of the neuronal architecture. HS in neurons leads to the induction of stress proteins which are involved in the degradation of misfolded proteins

and cytoskeletal organization, while HSPs associated with the ER or mitochondria are not affected.

The porcine outer blood-retina barrier as blood-brain barrier model *in vitro* 881

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Top. A human-related *in vitro* model of the blood-brain barrier (BBB) for preclinical substance profiling in the area of toxicology, neuropharmacology, immunology, and drug targeting.

Content. Intact non-disintegrated preparations of pig retinal pigment epithelium as representative barrier tissue. Integration of the tissue sheet as interface in a 2-chamber culture device. Functional characterization: application of test agents and analysis of retention/transport across the barrier. Quantitative analysis by HPLC/mass spectrometry.

Background. The general problem of primary cell cultures used as *in vitro* models is that the native BBB structure needs to be destroyed in order to isolate the relevant cells. During subsequent culturing of these cells, various BBB functions are not reconstituted. The alternative approach is to use a tissue that a) displays CNS relevant barrier and carrier systems and b) can be directly employed *in vitro* without prior disintegration. The only appropriate tissue is the outer blood-retina barrier which is formed by the retinal pigment epithelium (RPE) that surrounds the neural retina of the eye. As the pigment epithelium functions as the retinal-blood barrier, it features typical BBB characteristics, including many molecular, structural and functional components.

Results. 1) RPEs with and without retina were successfully isolated as intact tissue sheets. 2) RPE tissue sheets were integrated as interface into a 2-chamber system for transport studies; native barrier characteristics could be shown with fluorescent markers and non-penetrating pharmaceutical agents ($Pe<2x10^{-7}$ cm/sec). 2) Comparative permeability studies with 10 different pharmacological agents were performed: the RPE model displayed a high dynamic range and allowed to distinguish permeability coefficients (Pe) over 3 orders of magnitude. 3) P-glycoprotein (P-gp) and multidrug-resistance related protein (MRP)-like efflux-pumps were demonstrated employing specific ligands (calcein, verapamil, probenicid, Na-fluorescein) in a) pharmacological competition assays and b) by fluorescence microscopic image analysis of tissue whole mounts.

Conclusion. RPE tissue sheets promise to be a valuable blood barrier model in drug profiling and mechanistic studies.

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882 Different mechanisms of astrocytic calcium-wave propagation in cortex versus hippocampus

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Signalling with Ca^{2+} waves represents the long-range communication system of astrocytes. Ca^{2+} waves have been extensively studied in culture and can be elicited by mechanical or electrical stimulation or by a local application of ATP. They spread through a large population of astrocytes with a speed of about 10 to 20 mm/s. Studies in culture identified two mechanisms of propagation, ATP release and activation of purinergic receptors, and spread via gap junctions. We studied Ca^{2+} wave propagation in acute slices (postnatal day 10 to 14 old mice) from two different brain regions, neocortex and hippocampus. We used a paradigm to selectively load the Ca2+indicator Fluo-4 into astrocytes. Cells were identified by a (transgenic) astrocyte indicator mouse or by characterizing membrane currents with the patch-clamp technique. In control solution, electrical stimulation elicited a Ca^{2+} wave in the astrocyte population that propagated with a high speed of 200 µm/s in cortex and a much lowe speed of 20 µm/s in the hippocampus. The wave extended in average for 430 µm in cortex and 270 µm in hippocampus. Blocking either action potential propagation by tetrodotoxin or synaptic transmission by nominally Ca^{2+} free solution had a strong effect on the Ca^{2+} wave propagation, particularly in the cortex: Ca^{2+} signals propagated with a much lower speed of about 12 µm/s in cortex and and also in the hippocampus, comparable to the propagation in purified astrocyte cultures. Thus, blocking neuronal activity unmasks the intrinsic astrocyte wave and using Ca^{2+}-free solution allowed us to study this waves in slices. Blocking gap-junctions with carbenoxolon inhibited the spread of the astrocyte Ca^{2+} wave in the neocortex whereas it spread almost unaffected in the hippocampus. In contrast, the purinergic receptor antagonist suramin inhibited the spread of Ca^{2+} waves in the hippocampus (80 % reduction) but was less effective in the neocortex (50 % reduction). We therefore conclude that neuronal activity modulates the propagation of astrocyte Ca^{2+} waves and that astrocytes from different brain regions express distinct forms of long-distance communication.

883 Suppression of receptor-evoked calcium signaling and control of release function via elevated basal calcium levels in activated microglia

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Microglia are the immune-competent cells of the CNS and respond to pathologic events. In culture, microglia can be activated by bacterial lipopolysacharide (LPS). We used this model to study alterations in the basal intracellular calcium concentration ($[Ca^{2+}]_i$) as

well as in the receptor-evoked generation of transient calcium signals. LPS treatment of mouse microglia led to a chronic elevation of basal $[Ca^{2+}]_i$ in association with a drastic suppression of evoked calcium signaling, as indicated by reduced $[Ca^{2+}]_i$ transients upon stimulation with UTP and complement factor 5a (C5a). As seen for LPS, the combination of elevated basal and suppressed evoked $[Ca^{2+}]_i$ was also obtained with a microglial exposure to the calcium ionophore, ionomycin, while the calcium chelator BAPTA partially reversed the LPS activation-induced changes in $[Ca^{2+}]_i$. In addition, we evaluated a more general role of basal $[Ca^{2+}]_i$ lifting in microglial activation. BAPTA strongly reduced the LPS-induced release of nitric oxide (NO) and of certain cyto- and chemokines. In contrast, ionomycin-induced $[Ca^{2+}]_i$ elevation failed to trigger any release activity. Our findings suggest that chronic elevation of basal $[Ca^{2+}]_i$ participates in the adjustment of receptor signaling. Moreover, increased $[Ca^{2+}]_i$ is required but by itself not sufficient for an effective release of NO and certain cyto/chemokines. Elevation of basal $[Ca^{2+}]_i$ could thus prove to be a central event in the regulation of executive features in activated macrophage-like cells.

Control of Ca^{2+} oscillations in astrocytes *in situ* **884**

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Astrocytes do not generate action potentials but respond with complex Ca^{2+} signals to stimulation either by ligands activating transmitter receptors, mechanical perturbation, or neuronal activity. These Ca^{2+} responses can be confined to subcompartments of astrocytes or can spread over large distances within a large population of cells. Spontaneous Ca^{2+} elevations (or also termed oscillations) have also been observed in astrocytes both *in vitro* and *in situ*. Here we studied Ca^{2+} signals from astrocytes in cortical slices of 10 to 14 day old mice by using a paradigm to selectively load astrocytes with the Ca^{2+} indicator Fluo-4. Cells were identified by a (transgenic) astrocyte indicator mouse or by characterizing membrane currents with the patch-clamp technique. We observed that the probability of generating spontaneous Ca^{2+} oscillations did strongly depend on the rate of slice superfusion. Spontaneous Ca^{2+} signals were rare when continuously exchanging the perfusate, but started soon after the superfusion was switched off and persisted during recording time (up to 4 min). Oscillations appeared with a frequency of 1 to 4 per minute. Activity again ceased to control value after the onset of bath perfusion suggesting that the observed Ca^{2+} activity is controlled by an accumulating extracellular factor.

Superfusion with nicotinic acid adenine dinucleotide phosphate (NAADP+, 5 mM) mimicked the stop of the perfusion in triggering Ca^{2+} oscillations largely in the same population of cells. NAADP+ was first described as an intracellular calcium-releasing messenger found in sea urchin eggs. It is generated from NADP+ by the activity of the ectoenzyme CD38. Using a specific antibody against CD38 we were able to colocalize CD38 with astrocytes in the cortex. Indeed application of the precursor NADP+ also induced calcium oscillations. Thus, the NAADP+ system may play a role in spontaneous calcium oscillations of astrocytes.

885 Structural Basis for the Interactions of Myelin-associated Glycoprotein with its Binding Partners

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Myelin-associated Glycoprotein (MAG) is a member of the Siglec family of I-type lectins1. While all other known members of the Siglec family appear to be involved in the regulation of the immune system, MAG plays a role in the nervous system by maintaining the stability of axon-myelin system. Recent studies have provided evidence that MAG interacts with different integral axonal membrane components and extracellular matrix constituents [2-6]. These MAG-ligand interactions seem to depend on sialic acid recognition in several cases (GT1b, Fibronectin) while others appear to be only protein-protein interactions (Nogo-66 receptor, p75 neurotrophin receptor). In order to identify the structural basis for these interactions we plan to analyse the different binding modes by methods like plasmon resonance spectroscopy and ELISA.

We have generated soluble Nogo-66 receptor, a protein similar to the Nogo-66 receptor and p75 overexpressing cells. We also generated Fc-MAG chimeras (fusion of MAG extracellular domains to the Fc-portion of human IgG) with either the three outermost extracellular domains or with all five extracellular domains ($FcMAG_{d1-3}$ and $FcMAG_{d1-5}$, respectively) to locate the region of ligand interaction. Our attempts to create $FcMAG_{d1}$ chimeras failed, since a disulfide bridge between domains 1 and 2, typically found in Siglecs, appears to be essential for the correct folding of this MAG domain. This has been demonstrated by mutations of the cystein residues in $FcMAG_{d1-3}$ involved in this disulfide bridge. These mutations caused the protein to be degraded intracellularly. To differentiate between sialic acid dependent and independent binding we generated MAG-mutants of the sialic acid binding site in domain 1 ($FcMAG_{d1-3}$ R118A, $FcMAG_{d1-3}$ R118K, $FcMAG_{d1-5}$ R118A and $FcMAG_{d1-5}$ R118K).

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Allocation of secretory organelle proteins to EGFP-expressing astrocytes *in vitro* and *in situ* 886

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Astrocytes take an active part in information processing within the nervous system, acting as receptive as well as signaling elements. They directly respond to synaptically released messengers and communicate, via signaling substances, with neurons in a reciprocal manner. Increasing evidence suggests that astrocytes are endowed with a pathway for regulated release of messenger molecules. Astrocytes cultured from new born rodent brain express a variety of SNARE proteins and also of classical synaptic vesicle proteins. We investigated the possibility that the protein expression pattern of immature astrocytes cultured from new born brain differs from that of mature astrocytes and of astrocytes *in situ*. Experiments were performed with transgenic mice expressing the enhanced green fluorescent protein under the control of the human glial fibrillary acidic protein promoter. Using double fluorescence we show that green fluorescent astrocytes cultured from 1 d to 16 d old animals reveal an organellar association of the t-SNAREs SNAP-25 and SNAP-23, and of the synaptic vesicle proteins synaptobrevin II, SV2, and synaptophysin. Acutely isolated astrocytes were immunonegative for SV2 but after 12 h in culture intense SV2 immunofluorescence was associated with cytoplasmic organelles. Green fluorescent astrocytes in brain sections of 1 d to 16 d old animals revealed a strong punctuate immunofluorescence for SNAP-23 and cellubrevin and partially for SNAP-25 and synaptophysin. Our data suggest that culturing can rapidly alter the astrocytic expression of synaptic proteins but that the protein expression is invariant of the age of donor animals. Acutely isolated astrocytes as well as astrocytes *in situ* contain an organellar membrane compartment equipped with SNARE proteins. This is first evidence that astrocytes *in situ* contain organelles that could be involved in regulated exocytosis of messenger substances.

These observations imply that astrocytes contain vesicular storage compartments and employ molecular mechanisms of release resembling exocytosis in nerve cells.

Gp130-mediated activation of the JAK/STAT-pathway is necessary for activation of glial cells following optic nerve lesion. 887

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Ciliary neurotrophic factor (CNTF) and leukemia inhibitory factor (LIF) have been shown to be involved in regulation of neuronal survival and glial reactivity following

CNS lesions. These cytokines signal through multimeric receptor complexes, which as an obligatory component contain gp130. Cytokine mediated signal transduction results in phosphorylation of STAT3, which is then translocated to the nucleus to regulate gene expression. To better understand the role of these cytokines, we examined glial reactivity in mice with targeted mutations of CNTF, LIF or gp130 after optic nerve lesions. The optic nerve was crushed intraorbitally and tissue was analyzed for protein and mRNA expression after different survival times or perfusion-fixed and immunostained for morphological analysis. In the retina of CNTF-/- animals, glial activation following lesion was stronger as compared to wildtype animals and was paralleled by a more pronounced phosphorylation of STAT3, a key step in the signal transduction pathway for gp130-associated cytokines. Obviously other factors activate STAT3 and in turn upregulate GFAP in the absence of CNTF. Using quantitative PCR, we could identify LIF as one of the factors, which after lesion are upregulated more strongly in CNTF-/- mice. Accordingly, basal expression and lesion-induced upregulation of STAT3 and GFAP were dramatically reduced in animals lacking LIF, suggesting that LIF is involved in mediating glial activation. In mouse mutants, in which gp130-mediated activation of STAT3 is abolished, optic nerve lesion did not induce an upregulation of GFAP in the retina. Interestingly, microglial cells in gp130-mutants were also less strongly activated as compared to wildtype animals, suggesting that this signaling pathway may not only be involved in regulating macroglial reactivity but also that of microglial cells. In summary our results show, that lesion-induced glial activation in the retina depends on gp130-associated cytokines, signaling via the JAK/STAT-pathway.

888 Gap Junctions serving Intercellular Communications are stabilized under the Plasma Membrane by Direct Interactions with Drebrin

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Following a series of intracellular trafficking steps connexins are assembled into hemichannels (presumably during transport out of the Golgi) to be installed into the plasma membrane. When joining between adjacent cells functional gap junctions (GJ) form and serve the exchange of ions, metabolites and second messenger molecules. GJ mediate also electrical coupling between cells.

To find interacting proteins involved in the regulation of connexin function we expressed the C-terminal cytosolic part of connexin-43 as a GST-fusion protein. One of the bands identified from pull-down assays of mouse brain extracts turned out to be drebrin, previously known only as an acting-binding protein. Fluorescently tagged dre-

brin (Drebrin-YFP) was expressed in Vero cells and the behavior of Connexin43 (expressed as a CFP chimera) was analyzed in response to drebrin expression.

FRET (fluorecence resonance energy transfer) analyses applied to the Cx43-CFP/YFP-drebrin pair revealed their interaction in the distinct spots underneath the plasma membrane. Decrease of endogenous drebrin E levels by transfection with drebrin-si-RNA oligos induced scattering of the connexins into a perinuclear (Golgi) region and throughout the cytoplasm. Electrical coupling as well as dye transfer between cells was impaired in drebrin siRNA – treated cells. Deficiency of drebrin E induced by si-RNA also resulted in a decrease in the total cellular amount of connexin43, compared to control proteins. This indicates an increased, and possibly regulated, degradation of connexons when they are not involved in junction formation.

Oligodendrocytes during myelination and trauma in PLP-DSRED transgenic mice **889**

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In the central nervous system oligodendrocytes form myelin sheaths which enwrap axon fibers and insulate them from neighbouring cells. Visualization and long-term observation of living glial cells in more native environment such as acutely isolated brain slices would provide a significant step towards our understanding of brain development and repair.

We addressed this question by use of transgenic mice expressing the red fluorescent protein DsRED regulated by the oligodendrocyte-specific proteolipid protein (PLP) promoter. Crossbreeding of these with GFAP-EGFP transgenic mice (EGFP under the control of the human GFAP promotor) allowed us the simultaneous observation of both major glial cell types, oligodendrocytes and astrocytes, in the very same animal. The transgenic PLP-DsRED mice exhibit selective and high level expression of DsRED in oligodendrocytes of white and grey matter in all regions of the central nervous system. Confocal laser-scanning microscopy of brain slices obtained from mice of different ages revealed the characteristic multi-process morphology of oligodendrocytes. In the brain stem oligodendrocytes could already be detected at postnatal day 1, in the forebrain, however, red fluorescent cells were not found earlier than postnatal day 10. In acutely isolated brain stem slices obtained from early postnatal mice time-lapse recordings showed movement of small membrane appendages along oligodendrocyte processes. To investigate oligodendrocyte and astrocytes under trauma conditions we transected axon fibers within the forebrain of the double transgenic mice. Increase of red as well as of green fluorescence could already be detected at 3 days after lesion and persisted for more than two weeks along the transection wound indicating enhanced myelin and astroglial gene activation in the traumatized brain tissue. Interestingly, also cells expressing both, DsRED and EGFP, could be detected around the lesion suggestive of resting precursor cell activation due to disturbation of the tissue integrity.

890 Dysmyelination Caused by CRE-Mediated Inactivation of Cholesterol Biosynthesis in Oligodendrocytes

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Cholesterol is a major component of myelin. Nevertheless, its function in myelin is only poorly understood to date. For genetic targeting we concentrated on squalene synthase, the first enzyme strictly dedicated to the formation of cholesterol. The conventional knockout of squalene synthase has revealed the overall importance of cholesterol. Tozawa et al. (1999) showed that squalene synthase deficient mice die around midgestation. Besides substantial growth retardation mutant embryos show a variety of severe malformations in the development of the nervous tissue. To circumvent embryonic lethality we introduced loxP sites into the mouse squalene synthase gene (SQS), and crossed these squalene synthase floxed mice with CNP-Cre knock-in mice. In this way we have generated oligodendrocyte-specific null mutants for cholesterol biosynthesis.

These conditional mutants reveal a trembling phenotype that becomes apparant at about 10 days of age correlating with the postnatal onset of myelination, a phenotype that is well-known for mouse models with CNS dysmyelination. Similarly, the spinal cord of conditional SQS mutant mice appears to be almost devoid of myelin compared to control mice. Residual myelin can be observed in subcortical white matter. At least some myelin is found in the anterior commisure and corpus callosum. Least reduction of myelin is observed within the cortex as jugded by histochemical methods. This mouse model will provide the opportunity to analyze the consequences of different levels of cholesterol on myelin synthesis.

891 Two-Photon Imaging of Glial Cells in the Intact Neocortex

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High-resolution fluorescence images of glial cells were obtained in the intact neocortex using two-photon laser scanning microscopy. We used transgenic mice in which astrocytes are labelled by human glial fibrillary acidic protein (GFAP) promoter-regulated expression of enhanced green fluorescent protein (EGFP). Mice were anesthetized and individual astrocytes with their extensive fine processes could be imaged down to several hundred microns below the pia in some cases even through the thinned skull. Counterstaining the cortical vasculature by tail vein injection of Texas-Red dextran visualized how glial endfeet enwrap cortical blood vessels. In addition, glial cells were found to be rapidly (within 10 min) stained by brief surface-application of sulforhodamine 101, presumably by a specific uptake mechanism. In conclusion, these glia staining techniques promise to enable studies of glia-vessel and glia-neuron interactions *in vivo*.

G-protein-mediated activation of glial functions in the leech central nervous system

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The two electrically coupled giant glial cells, localized in the neuropil of each segmental ganglion of the leech Hirudo medicinalis, are equipped with a variety of transmitter receptors and ion channels, and are thus a potential target of neuronal activity. The giant glial cells respond to an increased spike activity of the Leydig interneurons and to a peptide of the myomodulin family, the presumed transmitter mediating the Leydig neuron-to-giant glial cell transmission[1,2], as well as to 5-HT with a marked hyperpolarization due to an increased K^+ conductance. Since Leydig neuron activity as well as myomodulin and 5-HT modulate specific behaviours in the leech, they might be mediators linking neuronal circuits underlying behaviours and glial functions.

Recordings from one of two giant glial cells, using the two-electrode-voltage-clamp technique, suggested that the glial responses to Leydig neuron stimulation, to the peptide myomodulin as well as to 5-HT, are mediated by G-protein-coupled receptors: Injection of GDP-β-S, the non-hydrolysable GDP analogue and inhibitor of G-protein-mediated responses, results in an irreversible decrease of the giant glial cell responses to all three stimuli. We have also attempted to mimic the neuronally evoked hyperpolarization of the glial cell membrane: Injection of the activator of G-protein-mediated responses, the non-hydrolysable GTP analogue GTP-γ-S, in its inactive (caged) form, into both giant glial cells had no effect on the glial membrane potential or stimuli-induced hyperpolarizations; however, uncaging GTP-γ-S by UV-illumination evoked a rapid and irreversible hyperpolarization of the glial membrane to –76 mV, which is close to the K^+ equilibrium potential. The GTP-γ-S-mediated hyperpolarization of the glial membrane was accompanied by a fall in the input resistance of about 30% and a rise in the relative K^+ conductance of approximately 50%. After uncaging GTP-γ-S, the stimulation of Leydig neurons and application of myomodulin or 5-HT did not induce any additional membrane potential shift. Studies are on the way to elucidate whether these transmitters or the GTP-γ-S-mediated stabilization of the negative glial membrane potential affect neuronal properties and synaptic communication.

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[1]Britz FC, Lohr C, Schmidt J & Deitmer JW (2002). Glia 382: 15-227.
[2]Schmidt J & Deitmer JW (1999). Eur J Neurosci 11: 3125-3133.

893 Glutamate-induced morphological changes in the guinea-pig retina

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Glutamate serves as main excitatory transmitter in the mammalian retina, and modulates neuronal activity of second and third order neurons. The corresponding synaptic contacts occur in the two plexiform layers.

We visualized the different cellular elements of the guinea pig retina by using a laser scanning microscope. Neurons appeared in the reflection mode whereas mitotracker orange fluorescence labeled the Müller cells, the radial glia cells of the retina. In retinal wholemount preparations, stimulation with glutamate (10 min, 1 mM) caused the neurons in the ganglion cell layer (GCL) to swell to 140% of control values. Due to the small size of synapses it was not possible to detect morphological changes in the signal-processing synaptic layers directly. Therefore, we monitored the changes in the cross-sectional area of Müller cell processes which were clearly detectable in the inner plexiform layer (IPL), as an indirect indicator of volume changes in adjacent synapses.

In the IPL, the application of glutamate caused a decline in the cross-sectional area of Müller cell processes to 60% of the control values. Presumably this indicates a swelling of the surrounding synapses, as diffusion measurements with real-time iontophoretic method using tetramethylammonium selective electrodes indicated a reversible decline, rather than an increase, in the extracellular space volume during glutamate application (Nicholson an Sykova, 1998).

The glutamate-induced effect could be prevented by the AMPA/kainite receptor blocker CNQX, and was mimicked by kainate. Na^+- or Cl^--reduced extracellular solution abolished the glutamate-induced shrinkage of Müller cell processes, suggesting an osmotic effect underling the morphological changes in the IPL. Indeed, none of the used inhibitors acting on microtubuli (nocodazole), actin filament function (cytochalasine D) or myosin light chain kinase activity (ML7) affected the shrinkage of Müller cell processes; furosemide which blocks Na^+ K^+ Cl^- cotransporter activity, was also ineffective.

Depolarization of the retinal tissue by elevation of the extracellular K^+ concentration to 50 mM also resulted in Müller cell shrinkage, indicative of synaptic swelling. Again, CNQX was a potent inhibitor, as well as the blocker of endogenous glutamate release, riluzole. This suggests an intraretinal release of glutamate and subsequent activation of AMPA/kainite receptors after the depolarization of neurons.

Among several neurotransmitters and neuromodulators tested, Dopamine was the only substance that amplified the effect of glutamate-induced morphological changes (i.e., Müller cell shrinkage) in the IPL, although less intense than glutamate or depolarization.

The importance of dopamine in light-dark adaptation processes of the retina suggests a possible functional role of the observed morphological changes in this process.

Nicholson C and Sykova E, Extracellular space structure revealed by diffusion analysis. Trends Neurosci 1998; 21: 207-215

Biomechanical properties of Müller cells 894

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Müller cells, the main glial cells of the vertebrate retina, are the only cells spanning its entire thickness. In addition to physiological functions in the support of neurons, Müller cells are thought to serve as structural framework of the retina. Furthermore, they are thought to be involved in the genesis of different diseases of the retina, with mechanical stress playing an important role. Thus, we investigated the mechanical properties of Müller cells and retinal neurons.

Biological tissues and cells are complex viscoelastic materials. In response to applied mechanical stress they behave to some degree like solids, i.e. they store energy and provide a spring-like, elastic response, but also have liquid-like properties, i.e. they dissipate energy through viscous flow. The type of behavior generally depends on the frequency and force with which the sample is mechanically stimulated. (Gardel et al., 2002)

The mechanical properties of living cells depend mainly on the cytoskeleton, which is composed of three main components: actin, microtubules and intermediate filaments. The exact composition of the cytoskeleton of a single Müller cell changes within the different retinal layers, suggesting different mechanical properties at different segments.

We analyzed storage modulus (elasticity) and loss modulus (viscosity) at different locations along Müller cells (endfoot, at the inner process, and at the soma) and of Bipolar cell somata by using atomic force microscopy (AFM) as well as single-particle-tracking microrheology.

Using the AFM-technique, which is sensitive to the force required to indent a surface, we got information about the local elasticity at these points. The storage modulus of the endfoot averages 2070 Pa, that of the process 2280 Pa, the Müller cell soma has an average storage modulus of 460 Pa and the bipolar cell soma of 660 Pa.

Single-particle-tracking microrheology quantifies in our case the local mechanical properties of living Müller cells by monitoring the Brownian motion of individual phagocytosed fluorescent latex beads. Particle tracking of microspheres imbedded in the cytoplasm revealed information about spatial distributions of viscoelastic moduli. The behavior of the beads at the region of the Müller cell endfeet is indicative of a vimentin

network, which is known to be located in the Müller cell endfeet as well as in the inner process.

In conclusion, we can state that Müller cell processes and endfeet showed higher elasticity (i.e. "stiffness") than their somata. Compared to bipolar cell somata, Müller cell somata were shown to be less elastic (i.e. "softer").

These differences may contribute to the shaping of retinal tissue. Our results suggest that the stiffness of Müller cell processes may enable them to function as "support pillars".

Gardel M.L., Valentine M.T., Weitz D.A.: Microrheology In: Microscale Diagnostic Techniques K. Breuer (Ed.) Springer Verlag 2002 (in press)

895 Early change in extracellular atp-induced responses and potassium currents of Müller glial cells in experimental retinal detachment: Effect of suramin

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Injury and diseases of the nervous tissue are accompanied by reactive gliosis of microglial cells and astrocytes. Reactive gliosis is often accompanied by a downregulation of the main plasma membrane K^+ conductance of astrocytes which is mediated by inwardly rectifying K^+ (Kir) channels; the inactivation of K^+ channels may result in failure of the extracellular K^+ homeostasis in the injured nervous tissue and was suggested to reflect an injury-induced dedifferentiation of glial cells as precondition for glial cell proliferation in the process of scar formation and reorganization of the nervous tissue. Activated glial cells alter their expression of functional receptors, and among other receptor types, activation of purinergic P2 receptors have been implicated in the induction and mediation of astrogliosis. In the present study, we investigated the time-dependences of the injury-induced downregulation of the inwardly rectifying K^+ (Kir) channels and of the upregulation of P2Y receptor-mediated calcium responses in Müller (radial glial) cells of the rabbit retina. Moreover, we investigated whether the injury-induced alterations could be blocked by suramin or by pyridoxal phosphate 6-azophenyl-2',4'-disulfonic acid (PPADS). Local retinal detachment was induced by subretinal injection of a sodium hyaluronate solution. Three, 24, 48, and 72 hours after surgery, Müller cells were acutely isolated, and patch-clamp records of the whole-cell potassium currents were made. The presence of P2 receptor-mediated responses was determined by extracellular ATP-induced current increases. At the vitreal surface of retinal wholemounts, ATP-induced calcium responses were recorded fluorometrically, and the density of microglial cells was determined. Within 24 hours of detachment, Müller cells begin to downregulate their inwardly rectifying potassium currents, with a decrease to 62 % of the control value at 48 hours of detachment. This downregulation was (with a similar time course) accompanied by an enhanced responsiveness of the cells to extracellular ATP, i.e. significant more cells showed calcium and current responses upon ATP application (control:

13 %; 24 hours detachment: 42 %; 72 hours detachment: 79 %). At 48 and 72 hours of detachment, cellular hypertrophy was apparent as indicated by the increased membrane capacitance of isolated cells. Application of suramin during surgery reversed the injury-induced down-regulation of potassium currents, the Müller cell hypertrophy, and the increase of the number of microglial cells in retinal wholemounts, while PPADS had no effect. The results show an upregulation of ATP-evoked responses in Müller cells within 24 hours of detachment, suggesting an involvement of P2Y receptors in early steps of Müller cell gliosis. Suramin application may be helpful to limit detrimental effects of Müller cell gliosis during retinal detachment.

Thrombin-induced ERK1/2 activation through PAR-1 in rat astrocytes is mediated by the Ca^{2+}-sensitive tyrosine kinase Pyk2 and Src kinase

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Astrocytes have recently been revealed to play a much more central role in maintaining the brain's vitality than researchers had thought for many years. Astrocytes function as an intimate partner of neurons to coordinate the activity across cellular networks in the central nervous system. Proteases like thrombin can act on astrocytes via activation of cell membrane protease-activated receptors (PARs), thereby mediate cellular responses such as morphological changes, proliferation and survival which may have important influence on neuronal development, plasticity and repair of injury. We have recently demonstrated that multiple signaling pathways are involved in thrombin-induced proliferation in rat astrocytes (Am. J. Physiol. Cell Physiol. 2002, 283: C1351-64). Thrombin acts by protease-activated receptor-1 (PAR-1) via mitogen-activated protein kinase (MAPK) activation. Mitogenic signaling is transduced through two branches: one is G_i/(β γ subunits)–phosphatidylinositol 3-kinase (Π 3-kinase) pathway, and another is G_q-phospholipase C (PLC)/Ca^{2+}/protein kinase C (PKC) pathway. In the present study, we investigated the possible protein tyrosine kinases which might be involved in thrombin signaling cascades. We found that in astrocytes thrombin can cause phosphorylation of proline-rich tyrosine kinase (Pyk2) via PAR-1, which is a subsequent event to the increase in intracellular Ca^{2+} and PKC activity. Moreover, in response to thrombin stimulation Pyk2 formed a complex with Src tyrosine kinase and adapter protein Grb2, which both could be co-precipitated. Furthermore, both thrombin-induced Pyk2 phosphorylation and extracellular signal-regulated kinase (ERK) 1/2 phosphorylation can be attenuated by Src kinase inhibitor PP2. From these data we conclude that PAR-1 utilizes Ca^{2+}- and PKC-dependent Pyk2 to activate Src, thereby leading to ERK1/2 activation, which predominantly recruits Grb2 in rat astrocytes. Supported by Deutsche Forschungsgemeinschaft.

897 Neuro-glial contacts and changes in the glyco-landscape of the cell surface.

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Cell surface glycoproteins mediate information transfer from the cell external into the cell internal world. Such information transfer involves translation of surface binding events, e.g.of diffusible ligands (ligand - receptor relationship), or of constituents of other cell surfaces or matrix molecules (intercellular and cell-matrix -receptor relationships) into intracellular signalling cascades. With rapidly increasing knowledge on common molecular pathways in cell-internal signaling cascades, questions on compartimentalization of information transfer and on "signaling value" have arisen. Together with cell-internal compartimentalizations, membrane compartimentalization of receptors provides a means for cell-type and event-characteristic signal transduction and functions. Here, we describe alterations in topographies of glial cell surface glycoproteins following neuro-glial contacts. As part of glial cell plasticity and differentiation, cell surface landscaping following establishment of intercellular contacts may be related to alterations in signaling compartments and values.

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898 Cytoplasmic Inclusions which Transiently Occur after Treatment with Okadaic Acid in Oligodendroglial Cells Overexpressing Tau Are Stabilized by Proteasomal Inhibition

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Filamentous inclusions in neuronal and glial cells, containing the microtubule associated protein Tau and a number of heat shock proteins (HSPs), such as alphaB-crystallin, are a common feature in a variety of late-onset neurodegenerative diseases. In normal brain, tau protein is a soluble and nonfilamentous protein. We have previously shown that oligodendrocytes, the myelin forming cells of the CNS, developmentally express all six isoforms of Tau, and that stress situations influence the degree of tau phosphorylation in oligodendrocytes (Goldbaum and Richter-Landsberg, 2002). The mechanisms underlying Tau deposit formation are still unclear, but Tau hyperphosphorylation, leading to the detachment from microtubules (MT), its cytoplasmic accumulation and subsequent aggregation has been suggested. Furthermore, malfunction of the proteolytic system may be causally related to protein deposit formation, and oxidative damage and decreased levels of proteasome activity might contribute to cell death during aging.

To investigate the consequences of Tau hyperphosphorylation, we have used the glial cell line OLN-93, stably transfected with the longest Tau-isoform (OLN-t40). Cells were treated with okadaic acid (OA), a selective inhibitor of the protein phosphatases PP1 and

PP2A. Immunoblot analyses with phosphorylation and site specific Tau antibodies demonstrate that OA led to hyperphosphorylation of Tau in several epitopes which was accompanied by the activation of extracellular signal regulated kinase ERK 1,2. OA treatment caused the detachment of τ from the MTs, and Tau-positive inclusions, which could be stained by thioflavine S (thio-S). These aggregates were specifically observable in the perinuclear regions. The inclusions were most prominent 6h after treatment and not detectable any more after 24h. The induction of alphaB-crystallin was not observed after OA alone. Treatment of OLN-t40 cells with MG-132, a proteasomal inhibitor, caused the induction of HSPs including alphaB-crystallin, the translocation of HSP60 and alphaB-crystallin to the perinuclear region, and the disorganisation of the MT-network, but not to the formation of insoluble thio-S- positive aggregates. However, OA-induced thio-S-positive aggregates were stabilized by proteasomal inhibition. These aggregates contain Tau and alphaB-crystallin.

Hence, hyperphosphorylation alone is not sufficient to induce permanent aggresome-like glial cell inclusions. Hyperphosphorylation followed by proteasomal inhibition causes the formation of stable aggregates, which initially might play a protective role, but eventually contribute to cell death.

Goldbaum O., Richter-Landsberg C.: Activation of PP2A-like phosphatase and modulation of τ phosphorylation accompany stress-induced apoptosis in cultured oligodendrocytes. Glia 40: 271-282 (2002)

Peroxynitrite Induces Cytoskeletal Changes and Cytoplasmic Inclusions in Oligodendroglial Cells Overexpressing the MAP Tau

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Nitrative and oxidative stress are involved in the pathogenesis of a number of neurodegenerative diseases, and the loss of oxidative stress tolerance has been linked with aging. Nitric oxide (NO) is a biological mediator released by a variety of cells. The reaction of NO with superoxide anion leads to the formation of peroxynitrite (ONOO-). Peroxynitrite (PN) is a strong oxidizing agent which attacks proteins by adding a nitro group on tyrosine residues. PN has been shown to interfere with signalling pathways and the mitochondrial electron transport chain. Increased 3-nitrotyrosine (3-NT) levels were identified in pathological cytoplasmic inclusions of neurons and glia in the brains of patients with Alzheimer`s disease (AD), Parkinson`s disease, Multiple Sclerosis and Multiple System Atrophy.

In the present study we have assessed the cytotoxic effects of PN on OLNt40 cells, an oligodendroglial cell line (Richter-Landsberg and Heinrich, 1996, J Neurosci Res 45: 161-173), which was stably transfected with a plasmid encoding the longest human isoform of the microtubule associated protein Tau. Tau modulates the stability and organization of microtubules, and is a major constitutent of neuronal and glial inclusions in various diseases, collectively called tauopathies. OLNt40 cells were directly treated with PN (0.5-1mM) for up to 24h. After 3h morphological changes were observed, cells

retracted their processes, and cell viability was reduced by 20%, as determined by MTT assay. Mitochondrial integrity was further studied by labeling cells with MitroTracker-Orange (MTO). In PN-treated cells MTO was almost not detectable indicating a break down of the mitochondrial membrane potential. Furthermore, lysosomes accumulated in the perinuclear region of the cells.

PN led to the nitration of a number of proteins including Tau, as investigated by immunoblot analysis, using antibodies recognizing 3-NT and nitrated Tau, respectively. Concomitantly, the small heat shock protein, HSP32 (heme oxygenase-1) was induced. HSP32 is an indicator of oxidative stress. While nitration of proteins was observable already 1h after treatment and increased with time, HSP32 induction occurred after 7h. Indirect immunostaining revealed that microtubule organisation was disturbed. PN led to the formation of intracelluar cytoplasmic protein aggregates which could be stained by thioflavin S.

Hence, peroxynitrite in oligodendroglial cells overepressing Tau causes an impairment of mitochondrial function, the disorganisation of the cytokeletal network, and the appearance of protein aggregates, indicating that nitrative insults contribute to pathological lesions and the death of glial cells, as observed in neurodegenerative diseases.

900 Neurons versus Glia -Differences in the transcriptomes of insect neurons and glial cells-

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The nervous system is made of primarily two different types of cells, neurons and glial cells. Both cell types could be divided in a number of different subclasses, each required for a certain task. Whereas neurons are required for information processing and information transport, glial cells are devoted to prepare the optimal conditions for the brain. Although both groups of cells are built of heterogenous groups of cells, some features should be specific both, for glial cells and for neuronal cells. Differences that are the basis for differential function are differences in the expression/transcription patterns of these two groups of cells.

To tackle this question, we used the fruitfly *Drosophila melanogaster* as the model organism. Like vertebrates, the nervous system of *Drosophila* contains both, neurons and glial cells. Glia in *Drosophila* could be subdivided into different populations such as wrapping or midline glia. For neurons the same heterogeneity could be observed. This holds true for the general function, e.g. interneuron versus motoneuron as well as for the use of specific transmitters or receptors e.g. cholinergic versus dopaminergic neurons. To allow a better understanding of the molecular rational that underlies these different functions we used the *Drosophila* Gal4/UAS-system that enables us specifically study single neuronal or single glial cell populations. For the first steps Gal4 lines were chosen that label either all neurons or all glial cells without subdivisions into one of the above mentioned groups of neurons or glial cells. The corresponding Gal4-lines used for these

experiments are based on insertions close to the repo (glial cells) and elav (neurons) genes respectively. Crossing of these lines with lines carrying a GFP under the transcriptional control of the UAS element allows to label the corresponding cell populations with GFP by simple crossing. After preparation of the nervous system and digestion of extracellular matrices, the cells could be isolated and subsequently analyzed and manipulated. To allow study of the transcriptomes of these cells, we isolated the GFP expressing cells using fluorescent activated cell sorting (FACS) and performed microarray hybridization experiments with these cells. Following amplification of the cDNA obtained from the different cell populations, two different chips were used for the transcriptomal analysis. The first is the Canadian 7k-chip that represent about 50 % of all *Drosophila* transcripts and the second one is a self made signal transduction chip, where about 600 genes involved in signal transduction (e.g. receptors, channels, molecules involved in second-messenger pathways) are deposited. The still ongoing analysis focuses on molecules of the signal transduction pathway that are differentially transcribed between glial cells and neurons.

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Differential expression of connexin mRNAs in the visual cortex of the rat

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Neurons are not single unconnected units but establish a neuronal network, and even a functional syncytium by direct connections through gap junctions. The gap junction proteins, the connexins, are numerous and divergent in their structure. This enables the system to connect cells by pores having different characteristics with regard to size or electrical polarity of the molecules passing through those channels. Neuronal network characteristics change with the functionality and activity of the system. In the rat, the eyes open postnatally and therefore the visual system matures only during postnatal development. With the input of visual information a number of well known characteristic changes occur in the visual system. We were interested if connexins are involved in these characteristic changes of the neuronal network. Therefore we investigated systematically the expression of the different connexin mRNAs (Cx26, Cx29, Cx30, Cx30.2, Cx30.3, Cx31, Cx31.1, Cx32, Cx33, Cx36, Cx37, Cx39, Cx40, Cx43, Cx45, Cx46, Cx47, Cx50, Cx57) in the visual cortex of the postnatal rat by using RT-PCR and the appropriate primers. We could show an expression of most of the connexins in the visual cortex of the rat and a differential expression in other neuronal and non-neuronal tissues.

The expression pattern of the connexin mRNA in the visual cortex changed for several connexins during maturation of the visual system, for others, the expression stayed more or less constant during postnatal development, some changed reciprocal others parallel in expression intensity.

We suggest that connexins which change in expression level in the visual cortex between pre and post eye opening might play a role in specific functions of the visual system.

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902 Proliferation and differentiation of neural precursors prepared from ventral mesencephalon of embryonic rats

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Dopaminergic neurones are generated between embryonic day 11 and 15 in the ventral mesencephalon of rats. Neural precursors, prepared from ventral mesencephalon at this early developmental stage, show a high self-renewal capacity, and therefore can be used for proliferation studies *in vitro*. Several groups have demonstrated that the basic fibroblast growth factor (bFGF) induces the proliferation of these precursors under serum-free conditions. The omission of this growth factor, and the cultivation of the precursors with fetal calf serum induce their *in vitro* differentiation into dopaminergic neurones.

In the present study, we investigated in detail the precursors under proliferation and differentiation conditions, respectively. Therefore, for one week, we cultivated the cells under following three culture conditions: with bFGF, FCS, and the combination of bFGF and FCS. To analyse changes in gene expression, we used the DNA microarray technique. With our newly developed microarray, we were able to measure changes in gene expression during the development of neuronal cells. The chip detects 350 relevant genes for neural specific proteins such as neurofilaments, receptors or neurotransmitter related enzymes. To further characterise the cultured cells, we carried out a proliferation assay with bromo-deoxyuridine (BrdU) and immunohistochemistry for the intermediate filament nestin, GFAP, and tyrosine hydroxylase. Approximately 60 % of cultivated cells expressed nestin under the three culture conditions. However, the number of BrdU-positive cells which expressed nestin was strongly reduced under the FCS condition. This data indicates that the expression of the precursor marker nestin is not correlated with the proliferation capacity. As expected, the highest number of tyrosine hydroxylase positive neurones were observed in the FCS group. Interestingly, under the culture conditions that combined bFGF and FCS, the effect of bFGF dominated over the FCS effects. These results were also confirmed by DNA chip experiments.

Enhanced sensitivity of accumbens core and shell neurons to dopaminergic drugs in adult rats with neonatal excitotoxic lesions to the medial prefrontal cortex

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Neonatal damage to the medial prefrontal cortex (mPFC) can lead, during adulthood, to abnormalities of both anatomy and function of meso-corticolimbic structures, including the mPFC, the ventral tegmental area (VTA) and the core and shell subdivision of the nucleus accumbens (NAC), which are associated with motor control and limbic functions.

Previous studies suggested that neonatal lesion of the mPFC increased the number of dopamine (DA)- D2 receptors in striatal and limbic areas and enhanced the sensitivity to DA agonists such as amphetamine. Therefore, in the present study, we investigated the effects of neonatal excitotoxic lesions of the mPFC on changes in firing rate in the NAC produced by the DA agonist apomorphine and the DA D2-antagonist haloperidol of core and shell neurons in adult rats.

Male pups received, on postnatal day 7, bilateral injections of either ibotenic acid (lesioned) or vehicle (sham-lesioned). Using extracellular single unit recording, we measured the spontaneous activity of core and shell neurons in adult rats. Injection of 4 mg/kg apomorphine produced a prolonged inhibition of neuronal activity that was significantly more pronounced in lesioned rats compared to sham-lesioned controls in both core and shell subregions of the NAC. The apomorphine-induced inhibition of the spontaneous activity was partially reversed by a subsequent intraperitoneal injection of 0,1 mg/kg haloperidol only in core subregion of lesioned animals but not in core and shell of control rats. No significant effects of the lesion on the discharge rate of either core and shell neurons were found.

This data suggest that neonatal excitotoxic damage to mPFC enhance the sensitivity of the meso-accumbal dopamine system that persists into adulthood and thus may alter the functional development and maturation of mesolimbic DA system. Since the shell region has been associated with limbic processes, wheras the core has been ascribed a primary role in motor control, our findings support the notion that a high D2 receptor and high sensitivity to apomorphine in core and shell is related to neonatal damage of the mPFC.

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904 A Novel Model System to Study Guidance Cues of Migrating Neurons

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Neuronal cell migrations play essential roles during the development of the nervous system. However, tools to observe or to manipulate migrating neurons are poorly developed. Here we present a novel mouse model that allows the visualization of migrating neurons via GFP- constructs and the simultaneous manipulation of guidance cues.

In this system, the Tet binary reporter system is used to drive expression of reporter constructs specifically in a subset of migrating cells. In a transgenic mouse line, the tetracycline-dependent transactivator (tTA) is expressed under the control of the human Pax6 promoter. The tTA is completely inert in vertebrates, however, enables the activation of artificial constructs containing the tTA-DNA-binding element (TRE). Reporter constructs can easily be introduced by electroporation and subsequently, the tissue can be sustained in an organotypic filter culture system for more than a week. Making use of the bidirectionality of the TRE, two genes of interest can be expressed from one construct, e.g. the green fluorescent protein (GFP) to visualize migrating cells and guidance receptor genes in order to influence their migration behaviour. Background activity in non-migrating cells is virtually undetectable. Focusing on tangential medullary migrations we present experiments demonstrating the influence of receptors of the netrin and slit pathways on these migrating neurons.

905 Guiding cues of tangentially migrating cells

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During embryonic development cell migration plays a crucial role in forming the anatomical shape of our brain. The precise location of a cell body in the central nervous system is essential for the subsequent elaboration of connections between individual neurons that enable their communication. To visualise migrating cells, we have generated transgenic mice which express the Tetracycline-dependent Transactivator (tTA) in all Pax6-positive cells. The tTA enables the activation of artificial constructs containing a tTA-DNA-binding element (TRE) and reporter genes, e.g. GFP. Plasmid constructs can easily be introduced into migrating cells by electroporation and subsequently, the tissue can be sustained in organotypic filter culture system for more than a week. Only in Pax6-positive, and therefore also tTA-positive cells, is the GFP reporter gene transcribed and can be visualised by using fluorescent light.

Cells of the posterior extramural migration stream (pes) of the rhombencephalon start their migration at the rhombic lip. After crossing the midline they settle in the lateral reticular and the external cuneate nuclei. Axons of these cells continue growing and

project predominantly to the ipsilateral cerebellum. Very little is known about the mechanisms controlling this migration and about the cues that guide these neurons. Using our tTA-reporter system to visualize migrating cells - the transcription factor Pax6 is specifically expressed in cells of the pes - and transplantation techniques we provide evidence that a combination of several external cues guide these cells to their correct place of settlement.

Functional maturation of the auditory cortex deprived from hearing experience

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Auditory deprivation leads to functional deficits in the organization of the auditory cortex in adult congenitally deaf cats (Kral et al., Cereb Cortex 2000, 10:714). Developmental plasticity in A1 field of congenitally deaf cats (CDCs) shows a sensitive period within the first 6 months p.n. (Kral et al., Cereb Cortex 2002, 12: 797). The present paper investigates whether these changes result from an abnormal postnatal maturation or from degenerative processes resulting from deprivation. Ten CDCs and ten hearing cats (ages 1 months - adult) were used. *Control animals* were normal hearing cats pharmacologically deafened at the beginning of the neurophysiological experiment. Stimulation with biphasic pulses (200 µs/phase) was applied by an intracochlear electrode (monopolar configuration). Local field potentials (LFPs) were recorded at 100-170 positions in the contralateral primary auditory cortex. Activated cortical areas (cortical areas with LFPs > 300 microV) were significantly larger at 8-12 weeks p.n. in CDCs, possibly indicating a cortical disinhibition at this age in deprived animals. The comparison of the shape of LFPs within activated areas showed no differences in maturation of P_a waves between CDCs and control cats. However, the maturation of N_b waves was delayed by 2 months in CDCs. Additionally, differences in P_b waves were observed. Long-latency P_1 waves were found in CDCs below 3 months age with similar shape and relative amplitude. In contrast to control cats, P_1 amplitudes substantially decreased with further age in CDCs. P_1 latency in young CDCs was smaller than in control cats.

The data demonstrate that cortical auditory maturation is substantially altered and delayed in auditory deprivation. In addition, degenerative processes set in at around 4 months p.n. The maturation can be caught up and the degenerative processes can be reversed in early implanted CDCs (Klinke et al., Science 1999, 285:1729).

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907 Chronic Modulation of Protein Kinase C Activity Affects Neuronal Connectivity in Cerebellar Slice Cultures

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Dendrite outgrowth is a crucial step in neuronal differentiation, regulated by various factors. In this context, protein kinase C (PKC) isoforms are of particular interest as they have been implicated in both structural and functional reorganization of neural networks. Morphologically, modulation of PKC activity alters the area and branching density of Purkinje cell dendritic trees. Increased PKC activity inhibits, while reduced PKC activity promotes dendritic growth (Schrenk et al., Neuroscience 2002 (110), 675-689).

Here, we report on the physiological consequences of PKC modulation in slice cultures of the mouse cerebellum. Slices obtained from the cerebellar vermis of P8 mice were cultured for 10-12 days as described recently. PKC activity was modulated by addition to the culture medium of either the PKC activator phorbol-12-myristate-13-acetate (PMA) or the PKC inhibiting indocarbazole Gö6976. We extracellularly recorded spontaneous spike activity with planar microelectrode arrays, covering an area of about 2-3 cerebellar lobes of the cultured slices.

On average, 27% of the 60 electrodes detected spike activity, mostly organized in several clusters. The firing rate of the recorded neurons varied between 0.2 and 25 Hz. For these properties of the spike activity, no difference was found between slices incubated under different PKC activity levels.

The connectivity within the slices was estimated from cross-correlations of the spike trains. Slices with pharmacologically enhanced PKC activity had a significantly reduced incidence of recording sites with correlated spike activity (34 ± 7 % of controls), while it was increased after PKC inhibition (144 ± 16 % of controls). Control experiments with acute application of the PKC-modulators did not show any short-term effects on neuronal connectivity, indicating that chronic modulation of PKC-activity is required to achieve the effects observed.

These findings are consistent with the expectations raised by the morphological data, insofar as reduced dendritic growth coincides with fewer inter-neuronal connections, whereas enhanced dendrite outgrowth and branching concurs with increased connectivity. Our results thus suggest a connection between the dendritic arborization pattern of Purkinje cells, including dendritic expansion and branching, and the functional characteristics of the cerebellar network as a whole, that directly of indirectly involves the PKC pathway. The potential contribution of other cerebellar cell types to these morphological and physiological alterations remains to be clarified however.

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Changing molecular complexities during ontogenesis in inferior colliculus and cerebellum of the rat brain

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A patterned histogenesis and the establishment of appropriate connectivity require differential expression and activity regulation of a large variety of proteins during ontogenesis of nervous tissue. In order to investigate the dynamics of molecular diversity during postnatal ontogenesis, we chose to compare the inferior colliculus (IC), a midbrain region serving auditory function, and the cerebellum (CB), a supervening motor structure of the rhombencephalon. We took tissue probes from rat brains at different ages (postnatal days 3, 6, 10, 15 and adult) and processed them to perform silverstained two-dimensional SDS-gel-electrophoresis (n = 3 to 8 per tissue and age). Protein spots were obtained from molecular weight 10 to 120 kDa and between pI 4 and 7. The number of spots was determined through softwarebased evaluation. Every spot in the gel stands for a protein or one of its posttranslational modifications, expressed in the specific tissue at a specific stage in postnatal ontogenesis. Comparing IC and CB, the spotnumbers showed significant differences along the course of maturation.

We found that the number of spots obtained from IC tissue started out at high levels (567 ± 33 S.E.M.), was held up to about the time of hearing onset, and decreases thereafter to settle on a significantly lower level at adulthood (413 ± 32; P3 + P6 vs. adult: $p = 0{,}0022$). By sharp contrast, spotnumbers from cerebellar tissue started out significantly lower than those from collicular tissue (343 ± 58; P3-CB vs. P3-IC: $p = 0{,}0357$) but increased thereafter to settle on a significantly higher level later on to remain there into adulthood (523 ± 35; P3 + P6 vs. P10 + P15 + adult: $p = 0{,}0175$), above the level of the IC (adult-IC vs. adult-CB: $p = 0{,}0426$).

To understand why the dynamics of molecular diversity in these cerebral regions are distinctly counter-regulated in postnatal ontogenesis, it may be helpful to realise that IC represents a region with sensory functions whereas CB is, with its major parts, integrated in motor functions. Until the onset of hearing by about P12, cells in the IC may maintain an indeterminate state regarding the function they will assume. Upon hearing onset, they turn functionally determined and rid themselves of the proteins and protein variations that underlay their pluripotentiality they have maintained until this developmental stage. By contrast, cells of the CB appear to set out on a reduced level of molecular variety, waiting to respond to a slow recruitment into elaborate motor programs to which they are not specifically predisposed molecularly. Whereas sensory-related neurons appear to be born with expectations as to the input they are about to experience, motor-related neurons may tend to develop increasing molecular complexity along the elaboration of motor programs that are to be learned with maturation.

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909 Expression and function of γ-protocadherins in the central nervous system of the mouse

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Many members of the cadherin superfamiliy are strongly expressed in the developing and adult nervous system. The functions of cadherin in the CNS include segregation of neuronal precursor populations and neuronal differentiation as well as axonal outgrowth and synapse formation.

Recently, more than 50 novel protocadherin genes were identified on human chromosome 5 / mouse chromosome 18 in an array of three clusters termed α,- β- and γ-protocadherins (PCDHs). The 22 γ-PCDHs are subdivided into A, B, C groups with 12, 7 and 3 members, respectively. These share the family-specific "constant" cytoplasmic domain, which is encoded by three exons located at the end of the cluster (Wu and Maniatis, 1999, *Cell* 97, 779-790).

We have investigated the expression of γ-PCDHs using *in situ* hybridization probes and an antibody directed against the constant cytoplasmic domain. We found abundant expression of γ-protocadherin transcripts in most neurons of the developing mouse brain and spinal cord. The strongest signals are observed, in neurons of the olfactory bulb, cortex and hippocampus, in cerebellar Purkinje cells. In glial cells γ-PCDH transcripts are absent. *In situ* hybridization for individual γ-PCDHs of the A, B and C groups yielded more specific expression patterns, similar to each other but with regional and cell type specific differences, indicating a differential expression of γ-PCDHs on the cellular level.

In Western blots, some γ-PCDHs are detected in embryonic stages. After birth, strong upregulation of γ-PCDHs is found with a peak during the second postnatal week. This expression parallels final neuronal differentiation and synaptogenesis in the mouse CNS. Indeed, γ-PCDHs are localized to synapse-rich areas, e.g., the cerebellar molecular layer, and after cell fractionation they are enriched in synaptosomes. Moreover, recombinant γ-PCDH A3, tagged with GFP is localized in synapses after transfection of hippocampal neurons.

To examine adhesive properties of γ-PCDHs in more detail, stable transfectants were established with Hek 293 epithelial cells. Transfected cells expressed the introduced γ-PCDHs at cell-cell contact sites and exhibited increased cell adhesion. Moreover, an increase of endogenous γ-PCDHs is observed in these cells. This suggests a molecular cross talk between transfected and endogenous PCDHs, possibly in heterophilic adhesion complexes, and / or transcriptional upregulation of the endogenous protocadherins by the introduced γ-PCDHs via retrograde signaling. In the future, the cell lines will be used to adress these issues.

In conclusion, our results support the view that γ-PCDHs may have important functions during neuronal differentiation, e.g., in generation of synaptic specificity which may be

modulated by the adhesion and signaling properties of differentially expressed γ-PCDHs.

Development of the serotonin 4(a) receptor isoform and co-expression with μ-opioid receptors in the pre-Boetzinger complex of rat **910**

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Recently we have shown that the 5-$HT_{4(a)}$ receptor isoform plays an essential role for the operation of the respiratory centre. Selective activation of these receptors by specific agonists protects spontaneous respiratory activity and overcomes fentanyl-induced respiratory depression. The 5-$HT_{4(a)}$ receptor (5-$HT_{4(a)}$ R) and the μ-opioid receptor (μ-OR) are members of the seven transmembrane-spanning G protein-coupled receptors, which stimulate (5-$HT_{4(a)}$ R) or inhibit (μ-OR) the activity of the adenylyl cyclase. Thus both receptors work in an opposite direction.

In this study we examined the co-expression of 5-$HT_{4(a)}$ R and μ-OR in the pre-Boetzinger complex (PBC) during development analysing prenatal (E14 - 20) and postnatal (P1 - 10) Sprague-Dawley rats. The PBC is located in the ventrolateral medulla and has been considered as the primary centre of respiratory rhythm generation. The PBC can be defined anatomically in adult rats by the surrounding cytoarchitectonic characteristics as the area ventral to the compact formation of the nucleus ambiguus, dorsal to the ventrolateral surface and directly rostral to the rostral border of the lateral and medial parts of the lateral reticular nucleus, and from a defined population of medullary respiratory neurons within the ventrolateral respiratory column expressing neurokinin-1 receptors (NK-1R).

Our immunohistochemical data indicated that the 5-$HT_{4(a)}$ R is predominately expressed in respiratory neurons, motoneurons of the brainstem and the ventral horn of spinal cord. We observed a strong labelling of these regions already during an early development stage of E14. In other upper regions the 5-$HT_{4(a)}$ R expression commenced arround postnatal day P10. A prominent staining of the facial nucleus, the hypoglossal nucleus and the ventral horn was primarily observed at embryonic day E14. Thus, we were able to demonstrate the first 5-$HT_{4(a)}$ R immunoreactivity of the region which evolves in the ambigual nucleus at embryonic day E16.

For the identification of interneurons such as respiratory neurons, we performed a triple labelling for 5-$HT_{4(a)}$ R, NK-1R and choline acetyl transferase (ChAT), the latter being a definite marker for cholinergic neurones such as motoneurons of the neighbouring motoric regions. NK-1R-IR was used for the localisation of the PBC. At early postnatal stages (P1 and P4) we detected non-ChAT-IR cells, which indicated a strong co-expression of both NK-1R and 5-$HT_{4(a)}$ R at the level of PBC. In prenatal stages, however, we did not find any specific NK-1R-IR in the region which evolves in the PBC at a later developmental stage.

Triple labellings with 5-$HT_{4(a)}$ R, μ-OR and ChAT indicated a strong co-expression of 5-$HT_{4(a)}$ R and μ-OR in motoneurons and in assumed respiratory neurons. To prove that 5-

$HT_{4(a)}$ R were expressed in respiratory neurons already at embryonic day E19/20, we used the patch-clamp technique to identify inspiratory neurons, which were filled with biocytin. These identified respiratory neurons revealed a strong 5-$HT_{4(a)}$ R-IR.

Taken together, our results indicate that the 5-$HT_{4(a)}$ R is expressed in the rat brainstem at early development stages and in identified respiratory neurons already at embryonic day E19/20 and is co-expressed with µ-opioid receptors.

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911 Analysis of Protein-Protein Interactions of Neuronally Expressed Basic Helix-Loop-Helix Transcription Factors.

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Transcription factors of the basic Helix-Loop-Helix (bHLH) protein family are well known for their instructive role in determining early cell fate decisions in the nervous system. The mRNA expression of the bHLH genes related to NeuroD are sustained in the adult central nervous system, in particular in areas known for their importance in learning and memory such as the hippocampus, the cortex and the cerebellum. The idea for a potential role of these factors in neuronal plasticity is supported by the fact, that the mRNA expression of at least one bHLH gene, SHARP-2, is induced by neuronal activity. bHLH-Proteins bind DNA as hetero- or homodimeric complexes, regulating the transcription of cell type specific target genes. The aim of this study was to analyze the protein-protein interactions of neuronally co-expressed bHLH transcription factors. We used FLAG-, HA-, Gal4- and VP16-fusion proteins for biochemical and reporter gene assays, respectively. Co-immuneprecipitation experiments were performed in COS1 cells. Reporter gene assays were performed in PC12 cells and primary cortical neurons to monitor protein-protein interactions and to analyze the transcriptional activity of different combinations of bHLH proteins upon depolarization. Using the biochemical approach, we found that the 'class B' celltype specific transcriptional activators NEX, NeuroD and NDRF interact with the ubiquitous class A transcription activator ME2 in this approach. In addition, we found that the 'class C' transcriptional repressors Sharp-1 and -2 can bind to ME2 and weakly to NEX, NeuroD and NDRF. Using the mammalian two-hybrid system, we could verify the constitutive bHLH domain dependent interaction between ME2 and NEX, NeuroD and NDRF in PC12 cells and in primary mouse neurons. The results of this work gives evidence on a crosstalk of different classes of bHLH proteins in neurons. Due to our analysis it appears possible, that the 'class A' protein ME2 could serve as a nodal point in transcriptional regulation of bHLH dependent target gene expression in neurons.

3D MRI of brain metamorphosis in *Manduca sexta* 912

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The moth *Manduca sexta* serves as a model for the neuronal development of the olfactory system. The pupal phase of *M. sexta* can be divided into 21 days of development (P0-P20) [1]. During this period males develop an antennal lobe with a neuronal architecture which enables the number of olfactory receptor cells to increase by a factor of about 10^4. So far magnetic resonance imaging (MRI) studies of insects did not involve the brain [2-6]. The purpose of this work was to establish MRI protocols for investigations of pupal brain metamorphosis.

Three-dimensional (3D) MRI was performed at 2.35 T (Bruker DBX) using a 100 mm Helmholtz coil for rf excitation and a 16 mm surface coil for signal reception. T1-weighted (FLASH, TR/TE = 20/7.8 ms, 25° flip angle, 8 averages, 3 h measuring time) and T2-weighted (FSE, TR/TE_{eff} = 3000/100 ms, 1 average, 3.5 h measuring time) 3D MRI data sets were obtained with a true isotropic resolution of 100 micrometer. Male *M. sexta* at different pupal stages of development (n=8) were investigated *in vivo*. To maintain physiologic conditions the coil set-up was tilted to allow for a horizontal positioning of the pupa within the surface coil. Anesthesia was not required. After 3D MRI the brain was removed for histologic assessment of the pupal stage.

Major cerebral microstructures such as the antennal lobe (AL), optical lobe (OL), and central brain (CB) of the sphinx moth *M. sexta* were clearly observable in both T1- and T2-weighted MR images. Shape and growth of various brain regions could be determined as a function of the stage of development during metamorphosis. About six days after pupal ecdysis (P6) first structures of the developing CB were identified. At P12 regions of the AL and OL could be distinguished from the CB. At later stages (P17) a better specific differentiation of the OL indicated a further cerebral maturation.

In summary, the present results demonstrate that both T1- and T2-weighted 3D MRI at 100 micrometer isotropic resolution are suitable for a determination of the cerebral anatomy with sufficient soft tissue contrast. However, T1-weighted 3D MRI might be advantageous, because it allows for an *in vivo* staining of brain structures with use of contrast agents [7]. Pertinent approaches offer potential for a specific delineation of neuroaxonal connectivity and architecture within the developing brain.

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913 Peripheral sensory neurons lead neurogenesis in trochophore animals

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The role of sensory inputs in development of the nervous system has been discussed for more than a century and yet remains unclear. Rapidly developing invertebrate larvae with simple nervous systems containing a small number of identifiable neurons provide a favorable model to address this problem.

Recent results challenge the generally accepted view on the early neuronal development in trochophore animals as undergoing neurogenesis beginning in the rudiments of central ganglia and then extending peripherally. It has been shown for representative species of trochophore animals that the very first neurons, which appear during the ontogeny, are peripheral FMRFamide and/or serotonin (5-HT) immunoreactive cells, and their fibers form scaffolding, upon which the adult central nervous system later develops. The nature of the first neurons and their roles in the development remain unknown.

We employed antibodies against the neurotransmitters 5-HT and FMRFa, and acetylated tubulin as a neuronal and ciliary marker, and confocal laser scanning microscopy (CLSM) to study in detail the first neurons in the chiton *Ischnochiton hakodadensis* (Mollusca: Polyplacophora), sea slug *Tritonia diomedea* (Mollusca: Opistobranchia), pond snail *Lymnaea stagnalis* (Mollusca: Pulmonata) and polychaete worm *Phyllodoce maculata* (Polychaeta: Phyllodocidae). In all the species, the very first neurons appear at the trochophore or early veliger stage, and are peripheral sensory (most probably chemosensory) cells. They always have a short dendrite bearing a tuft of cilia and an axon, which projects centrally and prefigures the layout of the future adult nervous system. Soon after these first neurons differentiate, a set of sensory neurons in the apical organ appears, and only upon that the larval nervous system and then the adult nervous system start to develop.

Pharmacological experiments have shown that monoamines released by the set of earliest neurons inhibit both growth of their own fibers and differentiation of following neurons. Induced overproduction of monoamines in these cells results in developmental arrest. In natural conditions, these neurons sense water-born substances released by conspecific juveniles and adults, and retard development in case of overcrowding.

Our results suggest that in the course of embryogenesis, sensory inputs including conspecific signals are essential for both the early stages of neurogenesis and normal passage of development.

TGF-β promotes survival on mesencephalic dopaminergic neurons in synergy with Shh 914

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Transforming growth factors β (TGF-betas) are widely distributed multifunctional molecules. In the developing and adult nervous system two isoforms of TGF-betas, TGF-β2 and TGF-β3, are involved in essential cell and tissue functions, including cell-cycle control, regulation of early development and differentiation, and neuron cell survival. Previous studies from our laboratory have provided evidence that TGF-betas promote survival on mesencephalic dopaminergic neurons *in vitro*. However, the molecular mechanism is not yet understood.

In the present study we investigated the survival promoting effects of TGF-β on mesencephalic dopaminergic neurons *in vitro*, using dissociated cells from the embryonic day (E) 14 rat midbrain floor. The results showed: 1) Treatment of the cells with TGF-β, increased the number of tyrosine hydroxylase (TH)-immunoreactive dopaminergic neurons after 7 days *in vitro*. 2) Neutralization of TGF-β in the presence of Sonic hedgehog (Shh) and fibroblast growth factor-8 (FGF8) or neutralization of Shh in the presence of TFG-β and FGF-8 induced reduction of TH-positive midbrain neurons. 3) Treatment of the cells with TGF-β, Shh and FGF-8 added together resulted to a significantly higher number of TH-positive neurons, compared to single factor treatments. 4) Neutralization of Shh did not effect initiation of TGF-β signal transduction, as shown by Smad translocation to the nucleus after treating the cells with TGF-β in the presence of neutralizing Shh antibodies. 5) Treatment of the cells with TGF-β in the presence of neutralizing anti-TGF-β antibodies, failed to induce Smad translocation to the nucleus. Taken together, these data suggest that TGF-β promotes survival on mesencephalic dopaminergic neurons acting in synergy with Shh

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Substrate- and concentration-dependent effects of nicotine on neurite outgrowth *in vitro* 915

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Accumulated evidence suggests that acetylcholine released from growing axons, regulates growth, differentiation, and plasticity of developing central nervous system neurons via activation of muscarinic and nicotinic acetylcholine receptors. Exogenous nicotine could also be a factor affecting neuronal development in the prenatal but also early postnatal period via the so-called colostrum-milk way in the mother-child relationship. The aim of this study was to clarify whether nicotine could affect development

of murine hippocampal neurons *in vitro* and to identify adhesion molecules involved in these effects.

Hippocampal cultures were prepared according to the method published elsewhere (Dityatev et al., 2000, Neuron 26:207-217). Cells were plated in 96-well plates at a density of 300 cells/mm^2 on glass coated with poly-L-lysine (PLL, 100 μg/ml) alone, or together with laminin (20 μg/ml) or fibronectin (20 μg/ml). Culture medium in some PLL-coated dishes was supplemented by NCAM-Fc (6 μg/ml) or L1-Fc (1 μg/ml) 4 hours after plating. The two latter chimeric molecules contain the extracellular domain of the neural cell adhesion molecule NCAM or L1 and the Fc portion of human IgG antibody and could stimulate neurite outgrowth via homophilic binding to neuronal NCAM and L1, respectively. Fibronectin and laminin stimulate outgrowth via different sets of integrin receptors. Four hours after plating, the culture medium was supplemented by 0.1, 1, 10, or 100 μm nicotine. Twenty hours later, the treated and control neurons were fixed and their longest neurites were measured.

Neurons grown on PLL and PLL+fibronectin coated glass coverslips showed shorter neurites in comparison to three other substrates. Neurite outgrowth on PLL and PLL+fibronectin was enhanced by nicotine in a concentration-dependent manner. For PLL, neurite outgrowth was 40-60% above control levels when the culture medium was supplemented by 10-100 μm nicotine. For PLL+fibronectin, it was increased by 40% with 10 μm nicotine. Neurite outgrowth on PLL+laminin was not affected by high concentrations of nicotine, but 0.1 μm nicotine inhibited it. Neurite outgrowth stimulated by NCAM-Fc was inhibited by 30% by 0.1-1 μm nicotine. The strongest inhibition of outgrowth by 40-60% at 0.1-100 μm nicotine was seen for neurons grown in the presence of L1-Fc, which maximally stimulated neurite outgrowth in the absence of nicotine. Thus, our experiments demonstrate both stimulating and inhibitory substrate- and concentration-dependent effects of nicotine on neurite outgrowth *in vitro*.

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916 Nitric Oxide and cyclic GMP mediated neuronal cell migration in the enteric nervous system of the grasshopper embryo

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Neuronal migration is a common feature of the development of the nervous system, since neurons are usually formed in specialized proliferative zones but ultimately reside in distinct locations where they function in the mature nervous system. The migration of a neuron is guided by the interaction between the neuron and its local environment. A novel aspect of cellular signaling during embryogenesis is the involvement of the membrane-permeant messenger molecule nitric oxide (NO). The dynamic regulation of nitric oxide synthase (NOS) and its effector enzyme soluble guanylyl cyclase (sGC) during the formation and regeneration of the nervous system has lead to the suggestion that

NO/cGMP signaling functions in developmental processes of both vertebrate and invertebrate nervous systems (1).

Here we investigated an example of neuronal migration in the enteric nervous system (ENS) of the grasshopper *Locusta migratoria* L. In particular, we focus on the directed migration of one set of enteric neurons, the midgut plexus neurons (MG neurons), which ultimately populate a branching nerve plexus that spans the midgut. The MG neurons arise in a neurogenic zone in the foregut, forming a packet of postmitotic neurons at the foregut-midgut boundary. Subsequently, they undergo a rapid phase of migration, during which the neurons cross the foregut-midgut boundary and travel several hundred μm posteriorly on the midgut surface (2).

The MG neurons exhibit NO-induced cGMP-immunoreactivity throughout the phase of migration and continue to show high levels of anti-cGMP staining in the phase of lateral axon branching and the formation of terminal processes on the midgut musculature. Moreover, we identified potential sources of NO near the MG neurons, in parts of the epithelial cells of the midgut using NADPH-diaphorase staining as a histochemical marker for NOS.

To investigate a potential role of the NO/cGMP signalling system during the development of the midgut plexus, we examined the pattern of migrating MG neurons in embryo culture. Using this *in vivo* culturing system, it could be demonstrated that pharmacological inhibition of endogenous NOS, sGC, and PKG activity results in a significant reduction of MG neuron migration.

This pharmacological perturbation of MG neuron migration can be rescued by supplementing with membrane-permeant cGMP and Protoporphyrin IX free acid, an activator of sGC, indicating that NO/cGMP signalling is essential for MG neuron migration.

In conclusion, both the immunocytochemical staining for cGMP and the pharmacological experiments in embryo culture are consistent with the hypothesis that NO/cGMP/PKG signalling regulates MG neuron migration in the ENS of the grasshopper embryo in a similar way as has been shown for pioneer growth cone extension(3).

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The role of c-ret signaling in the cholinergic differentiation of sympathetic neurons 917

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Development of the neurotransmitter phenotype in sympathetic neurons is a classical model for neuronal differentiation. In mammals and birds, noradrenergic and cholinergic populations of neurons can be distinguished in sympathetic ganglia according to their neurotransmitter, noradrenaline or acetylcholine, respectively. Whereas bone morpho-

genetic proteins (BMPs) have been demonstrated to be crucially involved in noradrenergic differentiation, the factors responsible for the induction of cholinergic differentiation *in vivo* are not fully characterized. In sympathetic ganglia of the chick embryo, cholinergic properties co-express with the growth factor receptor subunit c-ret, indicating that signal transduction via c-ret may be involved in the development of cholinergic sympathetic neurons. To test this hypothesis, we analyzed stellate ganglia in mice with a mutational inactivation of the tyrosine kinase domain of the c-ret protein.

Stellate ganglion size and position is largely normal in newborn c-ret mutant mice. Also expression of the noradrenergic marker enzymes tyrosine hydroxylase (TH) and dopamine β-hydroxylase (DBH) appears normal in the c-ret mutation. Expression of mRNAs for choline acetyltransferase (ChAT) and the vesicular acetylcholine transporter (VAChT), however, is strongly affected in mutant animals. The number of cells positive for these cholinergic markers is reduced by more than 80% in homozygous c-ret mutant mice in comparison with their wild type littermates. Whereas this reduction is apparent in neurons of sympathetic ganglia, expression of ChAT and VAChT mRNAs in spinal cord motoneurons of mutant mice seems to be unaffected. Similar observations are made in 16 day old mouse embryos.

Our results demonstrate that expression of c-ret is necessary for development of cholinergic sympathetic neurons in mice already at embryonic stages. The lack of cholinergic but not noradrenergic marker expression in sympathetic ganglia of animals with a mutation of the c-ret kinase domain indicates that c-ret signaling specifically affects cholinergic development. Apparently normal expression of cholinergic markers in spinal cord motoneurons points to specific differences in c-ret action between different neuronal lineages. Together with the co-expression of c-ret and cholinergic properties in chick sympathetic neurons, the data from mutant mice support the conclusion that c-ret signaling is required for diversification of transmitter phenotypes in sympathetic neurons of different vertebrate classes.

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918 The developmental change in the GABA response from depolarizing to hyperpolarizing

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During development, responses to GABA change from depolarizing to hyperpolarizing. Here we report a mechanism that limits the period during which GABA is depolarizing. Cultured midbrain neurons were loaded with Fura2-AM to monitor somatic $[Ca^{2+}]_i$ changes induced by GABA (50 mu M, 15 s) at different developmental stages. The L-type Ca^{2+} channel agonist FPL 64176 (1-3 mu M) was used to increase resting $[Ca^{2+}]_i$. Under these conditions the percentage of neurons that responded to GABA with an elevation of $[Ca^{2+}]_i$ decreased while the number of neurons responding with reduction in $[Ca^{2+}]_i$ increased with age. This developmental switch occurred even if $GABA_A$ receptors were permanently blocked during development. The decline of depolarizing GABA

responses could be mimicked by repetitive applications of GABA or through prolonged exposure to nanomolar GABA concentrations. These findings suggest that GABA irreversibly depleted Cl^- from immature neurons. In contrast, $[Ca^{2+}]_i$ reductions in response to repetitive GABA applications remained constant. Inward transport did not contribute to reestablish depolarizing GABA responses, however, the decline could be reversed by prolonged application of KCl (20 mM, 8 min). We conclude that passive equilibration of Cl^- across $GABA_A$ or glycine receptor channels can limit the period during which GABA is depolarizing. On the other hand, K-Cl cotransport can accomplish the change to hyperpolarizing GABA responses without involvement of $GABA_A$ channel activation.

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Glucocorticoid Induced Alterations of Brain Cytoskeletal Proteins in the Fetal Sheep Are Reversible after one Course of Drug Administration

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OBJECTIVE Synthetic glucocorticoids have been used for three decades to stimulate fetal lung maturation in human pregnancy at risk for preterm delivery. Previously we have shown that betamethasone (BM) at the dose clinically used in perinatal medicine to enhance fetal lung maturation results in an acute loss of microtubule associated proteins (MAPs) (1) and functional neuronal disturbances (2) in the fetal sheep brain at 130 day of gestation (dGA, corresponding to 32 weeks of gestation in the human fetus). MAPs are key proteins in brain morphogenesis and function . In the present study, we studied 1) whether the alteration of MAPs are independent of the prepartum surge of endogenous plasma cortisol level and 2) whether these alterations are reversible.

METHODS Chronically instrumented fetal sheep received 3.3 $\mu g\ kg^{-1}\ h^{-1}$ body weight betamethasone i.v. (n=8) or an equal volume of vehicle (saline, n=7) over 48 h. The betamethasone dose used produces fetal plasma betamethasone concentrations similar to those reached in the human fetus during antenatal glucocorticoid therapy (3). Fetuses were delivered by Caesarean section immediately after the end of betamethasone infusion (n=4) or 24 h later (n=4). Fetal brains were perfusion-fixed with 4% paraformaldehyde and embedded in paraffin. Tissue sections of the frontal neocortex, caudate putamen and hippocampal formation were stained with monoclonal antibodies against MAP1B and MAP2. Slices were counterstained with hematoxylin to visualize neuronal necrosis. MAP1B and MAP2 IR were quantified morphometrically.

RESULTS At the end of betamethasone infusion MAP1B and MAP2 IR were reduced in all brain regions investigated similar to the loss of MAP IR previously shown at 130 dGA. MAP1B IR was diminished by 45.3 % and 46.5 % in the frontal neocortex and caudate putamen, respectively ($p<0.05$). MAP2 IR was reduced by 51.8 % in the frontal neocortex ($p<0.05$), 67.8 % in the caudate putamen ($p<0.01$) and 28.8 % in the CA1

region of the hippocampus ($p<0.06$). Loss of MAP IR was not accompanied by neuronal necrosis.

24 h after the end of betamethasone administration, we could not show differences in both MAP1B and MAP2 IR between the vehicle and betamethasone treated group.

CONCLUSIONS Betamethasone induced structural alterations of cytoskeletal MAP1B and MAP2 occur independently of the prepartum surge of endogenous cortisol level and seem to be reversible within 24 h.

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920 Programmed cell death and maturation of glucocorticoid receptors are not related during brain development in fetal sheep

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Objective: Programmed cell death (PCD) is assumed to play a critical regulatory role in the developing CNS. In rats, PCD was found during the first weeks postnatally. There is little information of PCD in gyrencephalic brains that develop mainly prenatally. Studies in the adult brain have shown that selective occupation of type II glucocorticoid receptors (GR) induces apoptosis. This study was undertaken to examine 1) the time course of PCD in fetal gyrencephalic brains and 2) whether incidence of PCD is related to the development of GR in utero.

Methods: Number of apoptotic cells and GR expression in the fetal sheep brain were estimated at 40 (n=9), 60 (n=7), 80 (n=4), 110 (n=8), and 130 (n=3) dGA. PCD was investigated using the TUNEL method. GR were stained using a monoclonal antibody against GR (Clone BuGR2, Affinity Bioreagents Inc) and the avidin-biotin-peroxidase complex method. PCD was expressed as number of DNA-fragmented cells/1000 cells in the parieto-temporal cortex, thalamus, hippocampus and subcortical white matter. Density of GR was quantified using an image analysis program in the same regions.

Results: PCD was generally low in all brain regions with a maximum at 40 dGA ($p<0.05$). A peak of PCD was found in the white matter at 110 dGA ($p<0.05$) coincidencing with the peak of myelination (Dobbing and Sands, Early Human Develop 1979, 3: 79-83). GR could not be detected at 40 dGA. Expression of GR started first in the cortex at 60 dGA. GR could be detected from 80 dGA onwards in the other brain regions. Density of GR increased continuously until 110 dGA in all brain regions ($p<0.05$). There were no major differences in GR expression between 110 and 130 dGA.

Conclusions: PCD decreases with brain development whereas density of GR increases. We conclude that GR are not necessary to promote PCD during brain development.

Immunocytochemical localization of IGL, a new GAP-43 like gene product in different developmental stages of the American cockroach. 921

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Precise connection between single neurons is a prerequisite for the development of the nervous system. In vertebrates the growth associated protein GAP-43 is involved in the establishment of this complex network. The protein is transiently expressed in growth cones of immature neurons during axon elongation. In mature neurons with synaptic plasticity it persists in presynaptic areas. A GAP-43 related gene (*igl*, invertebrate GAP-43 like) was found in *Drosophila*. It encodes two proteins (IGLOO-L and IGLOO-S) with sequence homology and similar biochemical activity (Neel and Young, Development 120, 2235, 1994). In this study we report the cloning of an *igloo*-homolog in the American cockroach, *Periplaneta americana*. We investigated the expression pattern of an IGLOO-homolog protein in the cockroach at the level of single identified neurons by immunocytochemical techniques (double staining with antibody against perisulfakinin).

Cloning of *igl* from the cockroach was accomplished in RT-PCR experiments, using mRNA isolated from neuronal tissue. As for *Drosophila*, we found two putative *igl* mRNAs (*igl-L* and *igl-S*) in the cockroach. IGL-L is encoded by 193 amino acids of the larger mRNA. The *igl-S* mRNA encodes 118 amino acids and is identical to C-terminus of *igl-L*. IGL-L and IGL-S contain 3 and 2 putative calmodulin-binding domains (GAP-modules), respectively. Region of homology to *Drosophila* IGLOO encompasses the GAP-moduls and a putative N-terminal membrane-binding domain for IGL-L. The GAP-moduls are highly conserved repeats of the calmodulin-binding site from vertebrate GAP-43. Transfection of mammalian CHO cells with enhanced green fluorescent protein (EGFP) constructs confirmed membrane targeting for an IGL-L-EGFP fusion, but cytosolic localization for an IGL-S-EGFP fusion. For immunocytochemical studies of the membrane-bound IGL isoform we used a polyclonal antiserum raised against the aminoterminal domain of recombinant cockroach IGL-L in rabbits. Our results show that IGL-L is a nervous system specific protein expressed in neuronal cells of the brain (e.g. pars intercerebralis) and ganglia. Furthermore, immunostaining was found in all neuropil areas of the central nervous system, except for the central complex and mushroom body. IGL-L immunoreactivity was first visible in 18 day old embryos (50% of neurogenesis). During development, the number of stained cells and the intensity in neuropil areas increased. Whereas staining of neuropil persists throughout life, only a few cells show IGL-L immunoreactivity in adults. However, no IGL-L immunostaining was detectable in neuronal development of two protocerebral descending interneurons that show immunoreactivity against perisulfakinin (PDS neurons).

Furthermore, the antibody was tested in different cockroach species (*B. germanica*, *B. orientalis*, *P. australasiae* and *L. maderae*), *Drosophila melanogaster* and *Achaeta domesticus* with the result that only insects of the genus *Periplaneta* show immunoreactivity.

In PDS neurons, IGL-L was not detectable during axogenesis. This excludes a role in initiation of neurite extension. Its expression in late embryonic stages and the persistence in neuropil areas may, however, support a possible involvement in the establishment of synaptic connections.

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922 Thalamic Growth Cone Behavior Regulated by the Neurotransmitter Acetylcholine: Running on the Spot

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During the development of the nervous system, growing axons receive many signals from the environment that act as cues for the establishment of precise neuronal connections. These signals can mediate the guidance of individual axons, arrest their growth in the target region and induce synapse formation with target cells. Previous studies demonstrated that neurotransmitters, which are used in the mature nervous system for communication between different neurons, also play important roles in these processes during early developmental stages. For example, it has been demonstrated that acetylcholine inhibits axonal outgrowth of rat retinal ganglion cells (Lipton et al., 1989). In the present study we examined the possible role of acetylcholine on the development of thalamocortical projections. Using immunohistochemical techniques with an antibody directed against the vesicular acetylcholine transporter, we detected immunostaining in cortical structures of the mouse brain already at embryonic day 14. This suggests that acetylcholine is already present at early stages during the development of thalamocortical projections. Results from *in vitro* growth assays demonstrated that acetylcholine reduces the length of thalamic axons. The decrease in axonal length of thalamic neurons in presence of acetylcholine was dose-dependent, and a significant effect was already observed after application of 1 mM acetylcholine. To analyse the effects of acetylcholine in more detail, we used time lapse-videomicroscopy to examine directly the behavior of growing thalamic axons in presence of the neurotransmitter. Acetylcholine was applied from a micropipette positioned at a distance of about 100 µm away from the growth cone and 45° from the direction of neurite extension. A diffusion gradient pointing towards the thalamic axon was created by pulsed pressure ejections of the substance with a Picospritzer. We found that all thalamic axons stopped their growth within less than 2 minutes after acetylcholine application. The growth arrest was not accompanied by a collapse or a retraction of the growth cone, rather lamellipodia and filopodia were continuously formed and retracted, but there was no forward movement of the growth cone and thalamic axons were running on the spot. This effect lasted for at least 1 hour after termination of acetylcholine ejections. These results demonstrate that acetylcholine is not only used in as a neurotransmitter in the mature nervous system, but also has

pronounced effects on growth cone behavior. Since thalamic axons in rodents stop their growth for several hours when they first reach the cortical intermediate zone (Skaliora et al., 2000), we suggest that acetylcholine is one of the signals which mediates this effect and therefore acts as a local cue in a decision region for thalamic fibers.

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Developmental changes of voltage-dependent Ca^{2+} influx in insect neurons and glial cells during metamorphosis

923

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The sphinx moth *Manduca sexta* has been used for various aspects of developmental neurobiology, including changes in the nervous system during metamorphosis. We have investigated changes of Ca^{2+} influx into neurons and glial cells in the antennal lobe (AL) at different developmental stages during pupal maturation. Ca^{2+} was measured in glial cells with Fluo-4 using two-photon microscopy, and in neurons using ratiometric confocal Ca^{2+} imaging with Fura Red. Ca^{2+} influx induced by 0.1 mM Carbachol (CCH) or 50 mM K^+ (50K) could be observed in both neurons and glial cells, and was pharmacologically characterized as mediated by voltage-operated calcium channels (VOCC). Ca^{2+} influx in both neurons and glial cells was significantly reduced by diltiazem, verapamil and Cd^{2+} (all 0.5 mM).

We investigated developmental changes of the Ca^{2+} transients during metamorphosis. In glial cells, VOCC-mediated Ca^{2+} transients were not found until the end of the developmental stage 5 when glial cell migration begins. Glial cells of all later developmental stages investigated showed Ca^{2+} transients. In contrast, VOCC-mediated Ca^{2+} transients in neurons were not subject to developmental changes. Additionally, in deafferented ALs, i.e. in the absence of ingrowing olfactory receptor axons, neuronal VOCC-mediated Ca^{2+} transients were not affected, while the development of glial VOCC-mediated Ca^{2+} transients was suppressed.

Glial cells which were positioned in the centre of the AL (presumably migrating glial cells) expressed larger Ca^{2+} responses than peripheral (presumably non-migrating) glial cells. Blockade of VOCCs showed marked affects on the development of the AL. Injection of verapamil into pupae of early stages impaired both maturation of the AL and glial migration, suggesting that glial cell migration towards the centre of the AL requires Ca^{2+} influx.

924 Guiding Cells with Light

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A better understanding of cell motility is a fundamental objective in many disciplines, such as neuroscience, cell biology, developmental biology, biophysics, and biomedicine. We have predicted that we can use weak optical forces to guide the direction taken by the leading edge, or lamellipodia, of a cell by biasing actin polymerization. We have calculated that the power of our Gaussian laser beam¹s resulting gradient forces are too small to trap, but sufficiently powerful to bias actin monomer and oligomer diffusion. We are therefore using light to control a genuine biological process, the polymerization-driven lamellipodia extension, in sharp contrast to the established technique of optical tweezers, which uses large optical forces to manipulate entire structures. Depending on the shape and speed of lammelipodia extension various cell types responded differently to optical guidance. Alternate models how the weak optical forces interact with the lamellipodia are also discussed.

925 Optical guidance of growth cones

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The control of neuronal growth is an essential tool in neuroscience, cell biology, biophysics, biomedicine, and is particularly important for the formation of neural circuits *in vitro*, as well as nerve regeneration *in vivo*. We have shown experimentally that we can use weak optical forces to guide the direction taken by the growth cone of a nerve cell. In actively extending growth cones, a laser spot placed in front of a specific area of the nerve's leading edge enhances growth into the beam focus, resulting in guided neuronal turns and enhanced growth. We also discuss strategies for controlled synapse formation and ask for the minimal neuronal network that displays neuronal complexity. Our results open a new venue to control neuronal growth with a simple, non-contact technique, with potential applications ranging from neural network studies in cognitive research, biocomputing, and peripheral nerve repair.

Emerging network organisation in compartment cultures of embryonic neocortex: (Mechanisms contributing to) GABAergic neurons distribution

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Neurons of dissociated embryonic rat neocortex (E16) were cultured in three compartments (2x7mm2) separated by a cell free space where neurites may grow freely. Compartments were limited by plating neurons in the presence of a silicone mask firmly adhered to the coverslip, which was taken off after adhesion of cells to the substrate. Adhered cells were allowed to proliferate for 4-6 days. After two weeks *in vitro* neurons inside each compartment have formed networks that were connected to the networks of the other compartments in the same coverslip. Abundant neuritic growth between compartments suggested that these became physiologically connected during the growth period. Labeling with lipophylic fluorescence marker (DiI or DiO) or with axonspecific antibodies showed that after one week *in vitro* a significant reciprocal innervation has been formed between the compartments. Ca^{2+} imaging confirmed that compartments are funcionally connected with each other. To check if the development of GABAergic neurons in these cultures were normal we immunolabeled 7-12 and 14 day old cultures with GABA antibodies. As expected a mixed population of larger and smaller GABAergic neurons could be identified among a larger population of non-GABAergic neurons. Unexpectedly, we observed many small GABAergic neurons in peripheral regions of the compartments, where only neurites but virtually no non-GABAergic cell bodies were present. Additionally inside compartments or in normal cultures small GABAergic neurons were most frequently positioned between groups of non-GABAergic neurons. This may indicate a preference for synaptic connections with distal regions of non-GABAergic neurons. We focussed our interest on the mechanism by which GABAergic neurons accumulate at and outside the periphery of the compartments. Small GABAergic neurons are generated in cultures after the 2nd DIV and we have already shown evidences that they migrate from original proliferation clusters to form a loose network superimposed to the network of non-GABAergic (de Lima &Voigt, 97). Thus, selective and active migration may be responsible for the peripheral distribution small GABAergic neurons. Alternatively it might be that following a unspecific period of proliferation and migration of young neurons, non-GABAergic neurons are eliminated from border regions. To approach this question we are currently following the development of neurons along the borders of single compartments in time lapse series (24hours intervals) during the first two weeks *in vitro*. After the last session cultures are immunolabeled for the retrospective identification of GABAergic neurons. Preliminary results indicated that GABAergic neurons became positioned outside the compartment network between the 9th and 12th day in culture suggesting a high rate of migration during this period.

927 Early onset of synaptic activity in Cajal-Retzius cells of embryonic mouse cerebral cortex

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Cajal-Retzius cells play an important role in the establishment of cortical lamination and are among the first neurons appearing during corticogenesis. The findings that they are capable to fire action potentials (for review see Mienville, 1999) and receive synaptic inputs at early postnatal ages (Kilb & Luhmann 2001, Radnikow et al. 2002) suggest that they may be integrated in neonatal cortical networks. Since Cajal-Retzius cells are the first neocortical neurons showing synaptic structures during prenatal development (König et al. 1975) we investigated whether these cells already receive synaptic inputs in a preparation of embryonic mouse cerebral cortex (embryonal day 16.5) using whole-cell patch-clamp recordings from visually identified and biocytin-labeled cells.

Spontaneous postsynaptic currents (sPSCs) could be observed in 33 % of 30 investigated cells. The sPSCs occurred at a low frequency (0.05 ± 0.009 Hz, mean ± SEM, n = 10 cells), had an amplitude of 22.0 ± 1.5 pA, a rise time of 6.1 ± 0.8 ms (n = 107 events) and their decay could be fitted with a monoexponential function using a time constant of 21.5 ± 1.3 ms. The sPSCs could not be divided in subpopulations according to their amplitude distribution or kinetic properties. All sPSCs were blocked completely by application of the $GABA_A$ antagonist bicuculline (100 µm), while the glutamatergic antagonists (±)-2-amino-5-phosphonopentatonic acid (APV, 60 µm) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 µm) had no significant effect on sPSCs.

These results demonstrate that Cajal-Retzius cells receive synaptic inputs mediated by $GABA_A$ receptors as early as embryonic day 16.5, suggesting that they are integrated in neuronal networks at early stages of neocortical development.

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928 A screen for genes controlling gliogenesis in *Drosophila*

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The developing central nervous system (CNS) of *Drosophila melanogaster* is a perfect model system to study the mechanisms of cell fate specificiation and differentiation. It is complex enough to serve as a model for many of the molecular, cellular and developmental functions of the vertebrate CNS, and simple enough for single cell analysis. Glial cells play a central role in the development of complex nervous systems. They fulfil several functions such as providing a scaffold for the correct migration of neurons and guidance cues for growing axons, they control neuronal proliferation and maintenance of the mature CNS structure. Furthermore glial cells have to find their positions within

the developing CNS in order to fulfil their specific functions. This migrational process, which follows opposite directions along the dorsoventral axis for certain cells, needs to be controlled precisely. Our aim is to find genes involved in glial cell development and to gain more insights into the mechanisms of glial cell specification and migration. Though glial cells are essential for normal formation and function of the CNS, only little is known about the genetics and the functional machinery underlying gliogenesis in *Drosophila.* A nearly complete description of the glial cell organization in the *Drosophila* embryo enables a systematic genetic analysis of glial cell development.

With the aim to identify genes controlling glial cell differentiation and migration, we have performed an EMS (Ethyle-methane-sulfonate) mutagenesis screen. We have designed a new srategy for an efficient selection of mutants using GFP as an *in vivo* marker. From 3160 mutagenised lines on the second chromosome we obtained 2128 lethal mutations. From these several hundred lines show an interesting phenotype and were kept for further analysis. We sorted these phenpotypes into different categories:

* general developmental defects:	168
* overall PNS or CNS phenotypes:	150
* disarranged glial pattern:	128
* less glial cells:	50
* more glial cells:	1

We present here the characterisation of mutations that affect either the number or the positioning of glial cells (migrational defects). This comprises a detailed morphological analysis of the mutant phenotypes as well as the genetic mapping of the mutated genes.

Involvement of the NO/cGMP signaling pathway in the development of the antennal lobe of the sphinx moth *Manduca sexta* 929

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The metamorphosing CNS of the moth *Manduca sexta* is ideally suited to study mechanisms of development and plasticity. We use this system to investigate the developmental regulation and role of the NO (nitric oxide) / cGMP (cyclic guanosine monophosphate) signaling pathway.

During a defined period of metamorphosis cGMP levels are specifically elevated in local interneurons of the antennal lobe (AL) [1]. As recently demonstrated, this increase of cGMP concentrations is mediated by activity from the antennal nerve via releasing NO, by biogenic amines and possibly by other so far unknown mediators [2]. Seven to eight days after pupal ecdysis, when the AL neurons increase their cGMP levels the olfactory receptor neurons show spontaneous electrical activity [3] and the formation of the glomeruli (= synaptogenesis) in the AL begins [4]. Due to this parallel we assume

that the NO / cGMP signaling pathway might be involved in activity-dependent synaptogenesis in the developing AL.

To test this hypothesis we interfered pharmacologically with the NO / cGMP signaling pathway and combined it with measurements of synaptic densities by immunodetection of the ubiquitous synaptic vesicle protein synaptotagmin. As shown previously antibodies against synaptotagmin are a useful tool to study synaptic development in *M. sexta* AL glomeruli [4]. At times, when cGMP levels are regulated *in vivo*, we injected inhibitors of the NO / cGMP pathway and three to four days later measured synaptotagmin in homogenates of the antennal lobes by using the ELISA technique. The experiments show that inhibitors of soluble guanylyl cyclase (ODQ) and NO-synthase (7-nitroindazole, L-NAME) decrease the amount of synaptotagmin in the AL compared to controls at a given developmental stage. These results suggest that NO and cGMP enhance the rate of synaptogenesis within the AL.

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930 Imaging structural plasticity and calcium dynamics in dendrites of hippocampal neurons during synapse formation

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During the formation of functional neuronal networks not only axons, but also dendrites undergo dynamic structural remodeling: processes are elaborated, maintained or retracted. Likewise, contacts between axons and dendrites are formed, some of which mature into synapses, whereas others are eliminated. Even though it is widely accepted that dendritic plasticity contributes to these processes of synapse development, it remains unclear how dendritic plasticity is regulated. Previous work in the developing retina showed that spontaneous, transmitter-induced local calcium transients stabilize single dendrites. However, it is not known whether there is a relationship between dendritic calcium signaling and the spontaneous motility of dendrites.

To investigate this issue, we used hippocampal slices from mice or rats at postnatal days 1-7. The slices were incubated in organotypic culture for 1-7 days. Neurons were labeled with calcium indicator dyes (Calcium Green-1 dextran or Oregon Green BAPTA-1 dextran) using the gene gun. We routinely co-applied a calcium-insensitive dye (Alexa-594 dextran) and acquired dual-wavelength time-lapse recordings with a CCD camera. This experimental design allowed us to determine changes in fluorescence ratiometrically and, therefore, clearly separate those fluorescence changes that were caused by changes of the intracellular calcium concentration from those due to changes in dendritic structure.

We found that hippocampal neurons generated spontaneous global and local calcium transients that resembled those in other neuronal cell types. The generation of local calcium transients, but not that of global calcium transients, was inhibited by 2-APB (100 uM) an antagonist of IP3-receptors, indicating that local calcium transients represent release events from internal stores. Simultaneous imaging of dendritic motility and calcium dynamics revealed an interesting correlation between these phenomena: The frequency of local calcium transients increased in dendritic shafts when filopodia emerged from them. The mean frequency of local calcium transients in a dendritic segment was 0.16 ± 0.07 /min (18 filopodia from 9 cells) during a 2 minute period before the onset of filopodial growth. During the growth phase the rate of local calcium transients rose significantly (0.78 ± 0.14 /min, $p < 0.005$). After the maximum extension of filopodia was reached (typically after 2 minutes) the frequency dropped to 0.31 ± 0.10 /min. This observation indicates that local calcium signals may regulate the structural plasticity of dendrites. We are currently testing this hypothesis by pharmacologically manipulating the frequency of local calcium transients.

Immunocytochemically Unique Neurons of the Median Domain Contribute to the Primary Axon Scaffold of the Grasshopper Brain

931

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We are interested in the development of the primary axon scaffold of the embryonic grasshopper brain. This scaffold forms the basis for much of the subsequent axogenesis which occurs during embryogenesis. A number of neurons contributing to this axon scaffold, such as the primary commissure pioneers (Ludwig et al., 2001) have already been identified by our group. In the present study we examine the ontogeny and subsequent development of a pair of cells (the so-called lateral cells, LC) located in the median domain (MD), a mesectodermal region of the grasshopper brain. The LCs appear as a bilaterally symmetrical pair of cells in the MD, at its border with the protocerebral cortex (PC), at about 28% of embryogenesis. Staining with Lucifer Yellow or DiI reveals no precursor cells for the LCs, suggesting that they delaminate directly from the mesectoderm. We have confirmed their mesectodermal and neuronal identity via double immunolabeling with antibodies against HRP and Mes3. Subsequent to delamination each LC directs an axonal growth cone laterally along the glial bound border of the PC on its respective side. Concommittantly (30%) the LCs express the cell surface GPI-linked lipocalin *Lazarillo*. These are the only cells within the MD at this developmental stage to express this antigen, making their subsequent development easy to follow. Intracellular staining and immunocytochemistry reveal that the axon growth cones of the LCs pioneer a fascicle of the primary commissure (1° com) in the grasshopper brain. The LC axons then exit the 1° com, turn posteriorly and fasciculate with neurons of the next Lazarillo-expressing cluster. The axon scaffold is therefore constructed via a step-by-

step fasciculation by such co-expressing neurons. Current experiments are aimed at revealing the molecular mechanisms regulating this directed axogenesis, and the role of the LCs in later brain development.

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932 Properties of Na^+ currents of neuronal progenitor cells

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Neuronal progenitor cells represents a promising treatment option for neurodegenerative diseases. A prerequisite for improving the therapeutic effects of transplantation is the knowledge about functional properties of the pluripotent cells during differentiation. So far progenitor cells are regarded to be non excitable.

We used the neuronal progenitor cell line ST14A established by stable retroviral transfection of primary cells from rat striatum using the temperature-sensitive mutant of the SV40 large T antigen. This enables a pointed change of cell differentiation by the shift of temperature. At the permissive temperature of 33°C the cells grow unrestrictedly and stay at the state of progenitor cells. Recordings of ionic currents of non differentiated cells were done at 67 cells using the whole cell mode of the patch-clamp technique.

At a holding potential of -70 mV we found a fast activating and inactivating inward directed current with various amplitudes and a maximum of activation at -20 mV, in a small subset of cells (8/51). This current is driven by Na^+ ions since a reduction of the extracellular content of Na^+ results in a diminished current (n = 3) and the current turns out to be highly sensitive against the classic Na^+ channel blocker Tetrodotoxin (1 - 10 µm, n = 7). Furthermore Ni2+ ions (1 mM, n = 2), which blocks most of the known Ca^{2+} channels do not change the current. The differences in amplitude are not due to a different cell size, represented by comparable cell capacitances (P > 0.1). By further investigating the current properties we observe a remarkable shift in the steady-state inactivation towards deeply hyperpolarised values (half-maximum activation at -94.3 ± 0.74 mV, n = 13), This is about 30 mV less than described in adult striatal neurons, whereas other properties like activation kinetics of the current are comparable to the adult situation. Using a 100 ms hyperpolarising prepuls of -120 mV we detected the Na^+ current in nearly all investigated cells. As a functional consequence these Na^+ currents generate overshooting action potentials upon depolarizing current steps as in adult neuronal cells, held at membrane potentials below -100 mV by DC current injections. The resting membrane potential in the progenitor cells under our *in vitro* conditions ranges from -37 to -49 mV.

In summary Na^+ channels are present in the membrane of embryonic progenitor cells, but remain non functional since they are habitually inactivated. The data indicate a new, yet unknown intracellular mechanism controlling the neuronal excitability during the embryonic development.

Isolation and cultivation of CNS neurons from postnatal mice 933

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Survival and differentiation of neurons depend on signals from glial cells. Recently, new forms of glia-synapse interactions (Pfrieger et al., 1997; Ullian et al., 2001; Nagler et al., 2001, Mauch et al., 2001) have been revealed by studies of retinal ganglion cells, which can be highly purified from postnatal rats by sequential immunopanning (Barres et al., 1988). So far, however, comparable studies of glia-neuron interactions in other types of postnatal neurons have been hampered by the lack of appropriate isolation procedures. We report here the establishment of a new procedure to isolate hippocampal neurons from postnatal mice. Immunostaining against cell-type specific markers showed that the neurons can be purified to >99.5%. The procedure yields about 10% of the neurons contained in a suspension of dissociated hippocampi and about 8000 neurons/mouse. Immunostaining against glutamic acid decarboxylase showed that the population consists of 30% of inhibitory neurons. To assess the survival and growth requirements, we cultured purified neurons under defined conditions in a minimal medium (Neurobasal/B27, Invitrogen/Gibco). Using a commercially available assay (Live/Dead, Molec. Probes), we found that after 5 days *in vitro* (DIV), the survival rate is close to zero (1 ± 1%, n=3). Addition of glia-conditioned medium enhanced neuronal survival up to 7% (4 ± 3%, n=3). This low survival rate indicates that additional components are required. We are currently trying to identify these components and we are testing whether the immunoisolation procedure can be used to purify neurons from other regions of postnatal mice. These new preparations should allow for a variety of studies on the differentiation, growth and survival of CNS neurons and their dependence on glial signals.

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An atlas for the determination of the biological age of cricket embryos (*Acheta domesticus*) using morphological features 934

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Environmental conditions influence the embryonic and postembryonic development of organisms. In the central nervous system, neurogenesis is severely affected by modifications of sensory stimulation. We use house crickets (*Acheta domesticus*) as an experimental model to study the influence of gravity on neurogenesis during embryonic development. Former studies have revealed that in this species the physiology of the position sensitive interneuron Ψ was modified during exposure to hyper- or microgravity (Horn et al. 2002, Adv. Space Res. 30:819). In addition, the cell cycle duration decreased during gravity deprivation in the mouse cortex (R.S. Nowakowski: his report during the "Neurolab Final-IWG Meeting", Washington, 1999). Assuming that altered gravity

affects the period of proliferation of neurons, we expect persisting changes (1) for the number of neurons due to altered cell cycle duration, (2) for the target projection areas, and (3) for the topographical arrangements of inter- and motoneurons, in particular for those neurons which are linked to the gravity sensory system. In fact, preliminary studies point to morphological modifications in some peptidergic neurons of the cricket embryo brain by a 13-day hypergravity exposure starting 24 hours after fertilization. - One prerequisite of this study is the exact determination of the embryonal stage at each step of the experiment. We have to know whether embryos of the 1g-control group were at the same developmental level as groups with hyper- or microgravity experience. In particular, before preparing the CNS for the visualization of identified peptidergic neurons by means of immunocytochemical methods we have to look for basic tendencies within the process of development of the embryo. For this purposes it was necessary to develop an atlas which correlates the biological age of the embryos with easily detectable morphological features. Here, we present a catalogue for morphological characteristics which are useful to determine the biological age of the cricket's embryo (*Acheta domesticus*). Not in all embryonic stages the same characteristics can be used. For example, in young embryos from the period between 25% - 50% of embryonic development, the shape and size of the legs in the thorax are clear markers. In embryos which have exceeded 50% of their embryonic life, the size and colorization of the eyes or the sclerotization of hairs can be used. Throughout embryonic development, the length of the antennae and cerci are useful markers for the age. With this atlas, we have the tool to determine the extent of acceleration or deceleration of embryonic development after exposure to modified environmental conditions.

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935 Serotonin levels in brains of juvenile lobsters, *Homarus americanus*, show a diurnal rhythm

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Serotonin is a major neuroactive substance in the crustacean brain (Elofsson, Z Zellforsch. 97, 323-350 (1969); Sandeman et al, J Comp Neurol 269, 465-478 (1988)). In lobsters, paired serotonergic dorsal giant neurons (DGNs) located in the deutocerebrum arborize extensively in the olfactory and accessory lobes, and fibers from this neuron also project to cell cluster 10, which contains the somata of olfactory and accessory lobe projection interneurons. Cluster 10 is also one area where lifelong neurogenesis of interneurons has been demonstrated (Harzsch et al, J Neurosci 19: 3472-3485 (1999)). Experiments in our laboratory have shown that the timing of neurogenesis is entrained by light. Peak proliferation occurs during the hours surrounding dusk, the most active time for lobsters (Goergen et al., J Neurobiol. 53(1):90-5 (2002)). Consequently, we have addressed the question whether levels of serotonin, thought to be a regulator of neurogenesis in the lobster brain (Benton and Beltz, J Neurobiol. 46:193-205 (2001); Beltz et al., PNAS 98(22):12730-12735 (2001)), might also be entrained by light. High performance liquid chromatography (HPLC) was used to quantify serotonin levels in the

brains of juvenile lobsters that had been entrained to a 12:12 light:dark cycle, followed by 3 days in constant darkness. Brain levels of serotonin were sampled every 4 hours over a 24- hour period. The results demonstrate that serotonin levels in the brains of juvenile lobsters fluctuate depending on the time of the day with a trough at dawn, and gradually increasing levels during the day to a peak at dusk. Additional experiments will explore the possibility of entraining serotonin levels with light, by reversing the light:dark cycle and repeating HPLC measurements. The relative intensities of immunocytochemical labeling also will be used to verify these data. Based on the facts that serotonin levels fluctuate with a diurnal rhythm and that the rate of neurogenesis is responsive to this amine, we propose that serotonin functions as an integral component of a light-regulated molecular pathway influencing neurogenesis of the projection neurons in cluster 10.

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Influences on the development of the honeybee brain 936

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The European honeybee (*Apis mellifera*) has developed an advanced system for nest temperature control. This highly elaborated thermoregulatory system is especially important for brood rearing. The brood itself is incapable of regulating its own temperature and, therefore, completely relies on thermoregulation conducted by workers. Studies have indicated that prepupae and pupae are very sensitive to temperature changes (Gontarsky 1957). Normally, the central brood nest is maintained close to 35°C with only slight deviations. Early studies have shown that temperature affects the duration of development, and stronger deviations from the optimal temperature result in morphological malformations and an increase in the mortality rate (Himmer 1927). Previous studies focused on temperature effects on the general morphology of honeybees. Up to now nothing is known about thermoregulatory effects on the development of the central nervous system.

In the present study we began to systematically investigate effects of the temperature during the pupal period on the morphogenesis of the honeybee brain. For the experiments, the combs were transferred in an incubator in the lab immediately after capping. The temperature of the incubator was set to constant temperatures between 32° and 36°C, and the temperature of brood cells was monitored continuously. Within this range, no outside malformations were detectable after hatching. After emergence of the adult bees, their brains were dissected and immediately put in fixative solution. The brains were processed with immunofluorescence techniques using synaptic and nucleic markers to label synaptic neuropils and cell nuclei in the brain. The sections were evaluated using a laser scanning confocal microscope and appropriate image processing software. One of the regions we focused on were the synaptic input regions of the mushroom bodies (MB), as these prominent neuropils have been implicated in learning and memory in the honeybee and other insects. We began to quantify various parameters of the dimensions and synaptic organization of the neuropil in the olfactory and visual input region of the MB calyx.

For worker bees, the results indicate an overlap between 100% of emergence, the shortest developing time and a maximal neuropile growth rate at a temperature around 35°C. Compared to 1 and 7 day old workers, we find an increase in the lip area and in the number of synaptic complexes in foragers. Preliminary results in honeybee queens indicate a difference in the overlap between the shortest developing time and the maximum in the neuropile growth rate. In young queens we found the largest neuropil in the olfactory lip area and a maximum in the neuropil growth rate at 33.5°C rearing temperature. In adult queens the volume of the olfactory input region decreased with increasing age, but the density of synaptic complexes increased. We conclude that thermoregulation of the brood during the pupal phase may have important consequences for the growth and synaptic organization of central neuropils in the honeybee brain.

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937 Identification of lesion-induced genes in the hippocampus: A role for plasticity-related genes (PRGs) in layer-specificity?

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The molecular mechanisms of layer-specific axon guidance in the hippocampus during development are still poorly understood. Removal of hippocampal afferents induces a layer-specific axonal sprouting response in the denervated zones. This reorganization process is a form of neuronal plasticity in response to CNS lesion. This capacity of change suggests that molecules involved in lesion-induced plasticity participate in the development of the respective structure. Here, we use a differential cDNA procedure to identify genes that may participate in adult neuronal plasticity. We cloned 96 cDNAs of genes induced in the hippocampus by deafferentation. One of these cloned plasticity-related genes (PRGs), 1-13-E9, encodes a lipid phosphatase-related protein. This lipid phosphatase-related protein (PRG-1) is induced in the hippocampus following lesion and during dentate gyrus development. Further studies revealed that the plasticity-related gene PRG-1 is expressed specifically in the brain. In neurons, PRG-1 is localized in the plasma membrane and prevents phospholipid-induced neurite retraction. Our data suggest that PRG-1 may provide a novel guidance mechanism in neurons for controlling phospholipid signalling.

Adaptive changes of dopaminergic and serotonergic interaction in the nucleus accumbens depending on epigenetic factors 938

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We recently described that the dopaminergic innervation of the nucleus accumbens (NAC) is affected by an early methamphetamine (MA) challenge and that rearing conditions interfere with these drug-induced alterations (1). Therefore we checked the effect of a single early MA injection and isolated rearing (IR) on adult serotonergic fibre density in the NAC. Male gerbils were assigned to either enriched rearing (ER) or isolated rearing and received a single dose of MA (50 mg/kg i.p.) or saline for controls on postnatal day (PD) 14. On PD 110 the serotonergic innervation of the right hemisphere was visualized immunohistochemically and serotonergic fibre densities were quantified along the rostrocaudal extend in the core and shell of the NAC.

No difference could be found between IR and ER. The early MA injection, however, led to significantly increased serotonergic fibre densities in the NAC core of both the IR and ER group (12,5% and 18,5%, $p<0,05$). In the NAC shell, the serotonergic innervation was increased in ER (14 %, $p<0.05$) but not in IR animals. In the above mentioned dopamine study we demonstrated exactly complementary results for accumbal dopamine innervation patterns (1): The dopamine fibre density was significantly decreased in the core of ER and IR animals (54%, $p<0,05$ and 43%, $p<0,01$) after MA intoxication whereas it was decreased only in the shell of ER (54%, $p<0,001$) but not IR animals.

In conclusion it seems likely that (a) the MA-induced serotonergic hyperinnervation is at least in part a response to the loss of dopaminergic fibres (= heterotypic sprouting; 2) and (b) environmental rearing conditions interfere with the drug-induced alterations in the maturation of serotonergic and dopaminergic innervation patterns especially in the shell of the NAC. The shift of balances between the two prominent monoaminergic neurotransmitters supports earlier results on the central role of the accumbal system in psychotic processes.

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2. Yamazoe et al., 2001, Dev Neurosci 23: 78-83

An early methamphetamine intoxication exerts region-specific morphogenetic effects on the maturation of the cortical serotonin (5-HT) innervation: Interaction with environmental experience 939

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Both methamphetamine (MA) and restricted rearing affect the structure and function of monoaminergic transmitter systems in cerebral cortex of rodents (1, 2). The aim of the

present study was to prove whether an interaction of both variables during maturation of the cortical 5-HT innervation may occur. Furthermore, probable region-specific effects should be detected. Therefore, juvenile gerbils were assigned to either semi-natural or restricted environmental rearing conditions and received a single dose of either MA (50 mg/kg i.p.) or saline on postnatal day (PD) 14. On PD 110 the 5-HT innervation was visualized immunohistochemically and the fibre density was quantified in prefrontal cortex, insular cortex, frontal cortex, parietal cortex, and entorhinal cortex of the right hemisphere. Early MA treatment of ER animals was found to cause an increase of the 5-HT innervation of 51%, 23%, and 63% in the medial prefrontal cortex, orbital prefrontal cortex, and entorhinal cortex, respectively. In contrast, MA decreased the fibre density in insular cortex (28%) of IR animals. Significant effects of postnatal rearing conditions were found in insular cortex and entorhinal cortex, where increases of the fibre densities of 29% and 53%, respectively, were detected under IR conditions. Both frontal motor cortex and parietal somatosensory cortex did not show any vulnerability to MA or environmental rearing conditions. In conclusion, the extent of the MA-induced distortion of the 5-HT innervation is region-specific and coincides with environmental rearing conditions. Psycho-motoric disturbances following MA-intoxication or restricted rearing have been published by several groups. These behavioural deficits may – at least in part – be due to functional maladaption of the 5-HT innervation of cortical (3, 4) and subcortical (5, 6) brain regions. The present study was supported by the deutsche Parkinson Vereinigung (dPV).

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5. Lesting et al., this issue.
6. Polascheck et al., this issue.

940 Restricted rearing causes overshoot maturation of 5-HT innervation in amygdaloid nuclei

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The amygdala has been critically implicated in the modulation of emotional and social behaviours. Furthermore it is widely accepted that serotoninergic neurons are involved in the regulation of fear and anxiety. Isolated rearing (IR) affects serotonergic function (1) and IR animals often show an anxiogenic behaviour profile. We therefore checked for anatomical effects of (IR) by immunohistochemically staining serotonin fibres and assessing fibre densities in the central nucleus (CEA), basolateral nucleus (BLA) and lateral nucleus (LA) in the amygdala of Mongolian gerbils.

IR led to significantly increased 5-HT fibre densities in the right CEA (30,5% ; $p<0,01$), in the right BLA (10,7% ; $p<0,05$) and in the left BLA (16% ; $p<0,001$). No effects were seen in the LA and left CEA.

Facit: The increased 5-HT fibre density in the amygdala of IR gerbils is consistent with previous publications that in rats IR reduced serotonergic function (1) and 5-HT hyperinnervation led to a decreased transmitter turnover (2). Since 5-HT regulates the senso-

ry input to the amygdala by inhibiting excitatory inputs from cortical and thalamic pathways (3,4) an altered innervation pattern of 5-HT fibers by IR might result in a disturbance of those emotional functions controlled by the amygdala.

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2. Jonsson et Hallman, Bibliotheca Anatomica 23 (1982): 76-92
3. Stutzmann et LeDoux, J Neurosci 19 (1999) : RC 8
4. Rainnie, J Neurophysiology 82(1) (1999): 69-85

The maturation of serotonin and acetylcholine innervation in the dentate gyrus is influenced by epigenetic factors

941

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Serotonin (5-HT) and acetylcholine (ACh) are two transmitters that play a crucial role in modulating the functional state of the hippocampus (1). We recently reported that environmental experience and pharmacological intervention are two parameters that affect the maturation of the hippocampal 5-HT transmitter system (2). The current study should prove whether epigenetic factors influence the maturation of ACh in the hippocampus. For this purpose the effect of impoverished rearing (IR) versus enriched rearing (ER) was compared in conjunction with postnatal methamphetamine (MA) treatment (50mg/kg i.p. on PD 14). The densities of immunostained ACh fibres were quantified in a septal and temporal plane of the left and right hippocampal dentate gyrus (DG) in young adult gerbils.

In the IR group ACh fibre densities were principally enhanced. Only the granular (G), the inner molecular layer (iM) of the left septal DG and the G of the right septal DG showed no differences. Likewise, after postnatal MA-treatment the ACh fibre densities in the ER group were prevalently increased. No alterations were found in the G and the outer molecular layer (oM) of the left septal DG, and in the G of the temporal plane of the right hemisphere. Remarkably, IR in combination with MA diminished the ACh innervation of the subgranular layer of the temporal DG of either hemisphere. In our previous study (2) we demonstrated nearly the same alterations for 5-HT fibre densities in the DG: Under IR condition the 5-HT fibre densities were increased in nearly all layers of the temporal plane of the DG. The MA-application in the ER group led to a fibre surplus in the septal and temporal plane with the temporal plane being much more affected. In contrast, IR condition combined with MA significantly increased the maturation of 5-HT fibre densities only in septal layers of the right DG.

In conclusion, the fibre densities of both transmitters were equally affected by epigenetic factors. In view of the close functional association of 5-HT and ACh in the hippocampus (1) it might be likely that a mutual compensation between these two transmitter systems occurs during brain maturation. Alterations within these two transmitter systems could lead to disturbances within hippocampal plasticity and hippocampus-related cognitive and emotional function.

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2. Busche et al., Dev Neurosci, in press.

942 Lesion induced enhancement of LTP in rat visual cortex is mediated by NMDA-receptors containing the NR2B subunit.

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In this study we investigated the role of NMDA-receptors containing the NR2B subunit in a model of enhanced long-term potentiation (LTP) in the surround of focal lesions in rat visual cortex. Infrared-laser lesions were induced in the visual cortex of rats (P21-25). After 2-6 days survival time coronal slices of the visual cortex were obtained using a standard protocol. Extracellular field potentials (FP) were recorded at different distances lateral to the border of the lesion in cortical layers II/III after electrical stimulation in layer IV. Slices were superfused with ACSF containing low Mg^{2+} and CNQX to pharmacologically isolate the NMDAR dependent component of FPs. Application of ifenprodil (3µM) is known to selectively block NR2B-containing NMDARs. Ifenprodil reduced the FP to 69.01±3.72%, mean±SEM (n=8) in control tissue. The reduction was significantly stronger (p=0.045) in slices from lesioned rats at distances of 2.0-3.2 mm from the border of the injury (54.45±4.58%, n=6). However, the ifenprodil mediated reduction of NMDAR-FP amplitudes was not signigficantly different from controls when recorded close to the lesion (0.75-2.0mm,to 71.62± 4.51%, p=0.71). Furthermore, at distances of 2.0-3.2mm we observed a facilitated LTP induced by standard θ-burst stimulation (at 2.0-3.2mm: 136.62± 4.30%, n=6; controls: 120.15±3.02%,n=7, p= 0.02). Finally the LTP experiments were repeated in the presence of ifenprodil in order to measure the relation of the lesion induced changes in the NMDAR composition and in the strength of LTP. To our surprise ifenprodil did not significantly change the LTP in control animals (to 112±3.65, n=6, p=0.14), but it reduced the strength of LTP in slices post-lesion (to 116.02±4.75, n=7, p=0.02). These data indicate that a lesion in visual cortex is accompanied by an increase in the fraction of NMDARs containing NR2B subunits, which mediates the facilitated LTP in the first week following a focal cortical injury (Supported by the DFG).

943 Effects of combined administration of FK 506 and Simulect on sciatic nerve regeneration

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Purpose: Preceding studies have shown that administration of immunosuppressives following allograft peripheral nerve transplantation expedite nerve regeneration. The aim of this study was to assess the regeneration under combined administration of FK 506 and Simulect and to compare it to single administration of FK 506 in a rat model.

Methods: One group with 10 rats (5 DA and 5 LEW) was investigated after grafting of a 1.5 cm segment of the sciatic nerve. Each animal was treated with Simulect immediate after operation and 4 weeks post OP and received continuous injections of FK 506. The functional recovery of the affected limb was assessed 4, 8, 12 and 16 weeks post OP using sciatic functional index (SFI) and measuring the ankle stance angle (ASA) in the midstance phase of locomotion. After 16 weeks rats were sacrificed and nerve grafts were treated with myelin antibodies. Both histological and functional findings were taken as a marker for nerve regeneration.

Results: Preliminary results from ASA and histology show that combined administration of FK 506 and Simulect further increases nerve regeneration in comparison to single use of FK 506 and untreated allograft control. Preliminary findings from SFI do not support this conclusion.

Conclusion: The results of this study suggest that administration of Simulect further increases the acceleration in nerve regeneration of FK 506.

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Upregulation of the chondroitin sulfate proteoglycan NG2 in the zone of denervation and sprouting following unilateral lesion of the entorhinal cortex. 944

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Entorhinal cortex lesion partially denervates the rat fascia dentata. This is said to induce sprouting of intact fibers from neighboring layers that invade the zone of the degenerating axons. However, recent *in vivo* and *in vitro* studies failed to demonstrate sprouting across laminar boundaries. Sprouting does occur, but it mainly involves unlesioned fiber systems terminating within the layer of fiber degeneration. These findings point to laminar cues that promote sprouting of fibers within the denervated zone while repelling other, adjacent fiber systems that try to grow into the denervated zone. A group of molecules that are likely to guide the sprouting process and that could be involved in the formation of laminar borders in the denervated fascia dentata are chondroitin sulfate proteoglycans (CSPGs). These molecules delineate boundaries for growing axons during development and, thus, could also be involved in the molecular processes underlying the postlesional re-patterning of the fascia dentata.

A candidate molecule which may regulate layer-specific sprouting in the partially denervated hippocampus is the CSPG NG2. It has potent neurite outgrowth inhibiting properties and is produced by glial precursor cells and macrophages. Moreover, it is an important growth inhibitory component of glial scars following brain injury. To correlate the distribution of NG2 with the time course of the sprouting response, the distribution of NG2 was studied in the fascia dentata of adult rats 1, 4, 7, 10, 14d, 4wks, and 6 months after ECL using immunocytochemistry. In unlesioned controls, a cellular labeling pattern of NG2-immunoreactive glial cells was observed throughout the molecular layer. Following lesion, increases in NG2 immunoreactivity were observed in the dener-

vated zone by day 1, and a maximum increase was observed between days 7 and 14. This increase was prominently observed at the single cell level. Individual NG2-immunoreactive cells showed an enhanced immunostaining of their processes. Using double-labeling with glial markers, it could be excluded that these cells are astrocytes. Taken together, these data demonstrate that the growth inhibitory CSPG molecule NG2 is enriched in a denervated zone following brain injury. They suggest, that NG2, together with other CSPGs and glycoproteins of the extracellular matrix plays an important role in the regulation of layer-specific sprouting following entorhinal cortex lesion. (Supported by the DFG).

945 Commissural/associational sprouting in the hippocampus after entorhinal cortex lesion in adult mice overexpressing the growth-associated protein CAP23

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After entorhinal cortex lesion (ECL) the outer molecular layer (OML) of the fascia dentata is denervated. In response to this deafferentation several fiber systems sprout and reinnervate the OML. However, only few commissural/associational (C/A) fibers invade the denervated area from the adjacent nondenervated inner molecular layer. In order to test whether the sprouting response of C/A fibers can be enhanced by overexpression of the growth-associated protein cortical cytoskeleton–associated protein 23 (CAP23), ECL was performed in transgenic mice overexpressing CAP23 in adult neurons. The expression of CAP23 mRNA as well as the axonal sprouting response was compared in control and CAP23 transgenic (CAP23tg) mice. Non-radioactive *in situ* hybridization demonstrated that the sprouting neuron population expresses the transgene. Laser microdissection in combination with qRT-PCR revealed a very high level of transgene expression in the area of origin of the sprouting neurons. Anterograde tracing as well as immunocytochemistry were employed to analyze the sprouting response at the light- and electron microscopic level. In comparison to controls, CAP23tg mice showed an increased axonal sprouting response after ECL: Whereas only some C/A fibers invaded the denervated OML in controls, an enhanced invasion of the OML was observed in CAP23tg animals. In addition, sprouting fibers were longer in transgenic mice. These data indicate that CAP23 expression determines the extent of the C/A sprouting response in the hippocampus after ECL. (Supported by the DFG).

Effect of differentiation stage on fetal dopaminergic precursors survival and integration after grafting in animal model of Parkinson's disease

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The adult brain and spinal cord have only very limited restorative capacity following acute or chronic disease processes, unlike the peripheral nervous system. Degeneration of neurons in the central nervous system following acute or chronic illnesses like trauma, stroke or neurodegenerative diseases (Parkinson, Huntington) leads to a loss of function that is currently not amenable to successful restoration by intrinsic brain repair mechanisms and/or therapeutic interventions like medication and brain surgery. Therefore the cell-replacement therapy holds great promises to overcome those obstacles by reconstitution of normal-like neuronal circuitry. Results of studies in rodents and non-human primates with Parkinson's disease have shown that intrastriatal grafts of fetal mesencephalic tissue, rich in dopamine neuronal precursors, reinnervate the striatum and ameliorate motor symptoms. In spite of very promising preliminary results these approaches rise a lot of unresolved problems – from the difficulties with obtaining appropriate amount of fetal tissue to the ethical controversies. The possible solution could be proliferation *in vitro* and pre-defferentiation of fetal precursors in order to augment the number of cells disposable for transplantation. Earlier studies have shown that cultured fetal mesencephalic tissue can be efficiently expanded and partially differentiated into dopaminergic neurons.

Applying the modified *in vitro* protocol, we have propagated ventral mesencephalic precursor cells obtained from embryonic day 11 (E11) rat fetuses in the adherent culture system on a laminin-fibronectin coating. This developmental stage correspond to the most pronounced proliferative capacity - nearly 45% of the cells incorporate bromodeoxyuridine (BrdU) during proliferation phase and following differentiation present the highest yield of tyrosin hydroxylase positive neurons (TH/TuJ 1 ratio about 40%). The precursors were cultivated for 6-7 days with the presence of basic fibroblast growth factor (bFGF), differentiated afterwards for 0-1 or 2 days *in vitro* supplemented with ascorbate-2-phosphate and 1% fetal calf serum (FCS) followed by implantation in the striatum of the unilaterally 6-hydroxydopamine (6-OHDA) lesioned rats. The animals were subjected to the test of drug-induced rotational asymmetry pre- and post transplantation combined with more specific behavioral tests in order to investigate both lesion efficacy and functional graft integration. Parallel *in vitro* assay based on the passages of the cells at the above mentioned days of diffetentiation phase was also performed for more careful insight into the differences in the fate acquisition process in culture and *in vivo*. For *in vitro* cell characterization immunocytochemistry against nestin, β-tubuline III (TuJ1), glial fibrillary acid protein (GFAP) and thyrosine hydroxylase (TH) was applied. The morphological and qualitative analysis of graft survival were visualized via TH immunohistochemistry.

947 Gabapentin-lactam: A new potential neuroprotective agent.

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Parkinson's disease (PD) is the second most common neurodegenerative disease, afflicting about 1% of people over 65 years old and 4–5% of people over 85. Early in the disease, dopamine(DA)containing neurons in the substantia nigra pars compacta (SNc) degenerate, leading to slower movements, rigidity, rest tremor and disturbances in balance. As the disease progresses, many patients develop cognitive dysfunction, including anxiety, depression and dementia. In contrast to other neurodegenerative disorders, a relatively good symptomatic therapy for PD does exist, consisting mainly of dopamine replacement and adjuvant surgical therapy that relieves most motoric symptoms. However, there is no proven therapy to prevent cell death ('neuroprotective') or restore degenerating neurons to normal state ('neurorestorative').

In this study a potential neuroprotective agent gabapentin-lactam (GBP-L) is evaluated. GBP-L is a by-product of the synthesis and storage of the anticonvulsant drug gabapentin (GBP, Neurontin™). Intramolecular lactamization of GBP results in the formation of GBP-L. Accidental findings have revealed very promising neuroprotective effects of GBP-L *in vitro*. In order to test GBP-L *in vivo*, mini-osmotic pumps were subcutaneously implanted in 25 female Sprague-Dawley rats. Two days after the implantation of the mini pumps, all rats were stereotactically operated, getting a unilateral, terminal and striatal 4-site-lesion with 6-hydroxydopamine (6-OHDA). Thirteen subjects received GBP-L (300 mg in total with a pump rate of about 21 mg/day for approximately two weeks) and twelve were served as sham controls. One week post lesion, a blood test for measuring the level of free plasma GBP-L, revealed very low concentrations of GBP-L which contrasted to pharmacokinetic expectations. To monitor the effect of GBP-L on the lesion, rotation testing was carried out two and five weeks post lessoning. Both groups showed apomorphine- and amphetamine-induced rotation, but no difference between the GBP-L and the sham treated animals was recorded. Quantitative TH-immunohistochemistry is in progress and will be presented.

In parallel, *in vitro* studies on cell suspensions derived from the vetral mesencephalic tissue of embryonic day 14 (E14) rat embryos are examined, in order to understand the biological effect of GBP-L on dopaminergic (DAergic) neurons and the relation of the dosage to the neuroprotective effect of GBP-L.

Upcoming experiments will investigate the correlation of the dosage of GBP-L to the GBP-L plasma levels *in vivo*, employing different types of osmotic mini pumps or direct injections. Furthermore, the evaluation of DA tissue concentrations with the HPLC-technique, and the morphometrical analysis of the brain-sections, will produce a much clearer picture of the neuroprotegtive effects of GBP-L on DAergic neurons.

Mechanisms of Functional Restoration of Skilled Limb Movements after 6-hydroxydopamine Lesion and Dopaminergic Grafts: Restoration or Compensation? 948

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Unilateral lesions of the medial forebrain bundle (MFB) induced by 6-hydroxydopamine (6-OHDA) in rats lead to permanent impairments of end point measures and movement performance in tests for skilled limb use. It has been shown that transplantation of fetal dopaminergic cells helps to improve on some aspects of skilled limb use. However, little is known about the mechanisms of graft-induced behavioural recovery in skilled limb movements. The purpose of the present project is to determine whether transplantation of dopaminergic (DAergic) neurons mediates recovery of skilled movements by rewiring the neural circuit for reaching movements, or whether transplants promote compensation of motor impairments. Thirty-eight female Sprague Dawley rats were trained in skilled reaching tasks to grasp and retrieve food pellets from a staircase or to extend their forelimb through a slot to retrieve individual pellets from a shelf located outside the reaching box. Furthermore, animals were trained to cross a horizontal ladder beam (rung walking task) to test skilled walking and limb coordination. Animals then were subjected to one of the following experimental groups. Eight animals served as unlesioned controls, 30 rats received a unilateral MFB lesion with 6-OHDA injected stereotactically. Thirteen weeks after the lesion, 15 rats with MFB lesion were transplanted with DAergic grafts from fetal ventral mesencephalon (embryonic day 14; two tracts, two deposits each; 400,000 cells in total) into the lesioned striatum and 15 received sham transplantation surgery. The severity of dopamine depletion in these groups of animals was evaluated by assessing rotational behaviour after apomorphine and amphetamine injections two and five weeks after the lesion and transplantation surgery. Testing in the skilled reaching tasks and the rung walking task continued throughout the experiment. At specific time intervals before and after the surgical procedures, video recordings were collected for analysis of qualitative data of movement performance. The videotapes were evaluated independently by two people who were blind to the experimental condition. At the end of the experiment, histological analysis of the brains was performed by immunohistochemical analysis of tyrosinhydroxylase (TH) staining, and nigral TH positive cells were counted to assess lesion severity and survival of transplanted cells. This study is in progress and further data will be presented at the conference. Taken together, mechanisms of graft-induced functional restoration of complex sensorimotor behaviour differ significantly from simple behavioural tasks like rotation. For this, the rat model serves as a highly useful tool.

949 Xenotransplantation of rostral migratory stream (RMS) - and olfactory bulb-derived cells into a rat model of Parkinson's disease

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Loss of dopaminergic (DAergic) neurons in the substantia nigra and the resulting deficits in DAergic neurotransmission in the striatum are pathophysiologically relevant hallmarks in Parkinson's disease (PD). Recently, the finding that neural stem / progenitor cells exist in defined brain regions of adult mammals raised hope to exploit these cells for neurotransplantation. Throughout life stem cells residing in the subventricular zone of the ventricles generate neuroblasts which tangentially migrate along the rostral migratory stream (RMS) to the olfactory bulb where they eventually differentiate into interneurons (tyrosinhydroxylase (TH) - and GABA-positive). In this study we addressed whether cells of the RMS may represent a new source for cell replacement in parkinsonian rats. Neuroblasts expressing ß-galactosidase were derived from juvenile LacZ transgenic mice (ROSA 26) and transplanted into the striatum of immunosuppressed rats which were unilaterally lesioned with 6-hydroxydopamine (6-OHDA) before. After three and five weeks animals showed a poor recovery in the apomorphine and amphetamine rotation tests. After sacrificing the animals immunostaining against TH, ß-gal and mouse-specific antibody M6 was performed. These preliminary results show that RMS-derived neuroblasts at least survive the transplantation. The next step in our ongoing study will be to transplant cells explanted directly from the adult olfactory bulb. Further groups will receive grafted cells from the adult RMS and from E13 ventral mesencephalon derived from GFP-mice. These findings should further promote the identification and characterization of DAergic neuron precursor cells.

950 Survival of olivocochlear neurons and their role in reorganisation processes in the rat auditory system after cochlear lesion.

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There is evidence that a unilateral cochleotomy does not only cause degeneration but also reorganisation in the auditory brainstem. However, the cells of origin responsible for the reorganisation are unknown.

In mammals, the olivocochlear system enables the central auditory system to exert control over state and action of the receptor cells in the cochlea. There is no corresponding link in the visual system. The cell bodies of the olivocochlear system reside in the

superior olivary complex (SOC) and send their axons into the cochlea. Three cell groups may be distinguished. Lateral olivocochlear neurons (LOC-neurons) in the lateral superior olive (LSO) project to the region of inner hair cells in the cochlea on the same side. Some LOC-neurons have axonal collaterals to the inferior colliculus. Medial olivocochlear neurons (MOC-neurons) in the ventral nucleus of the trapezoid body (VNTB) form presynaptic endings on the outer hair cells on both sides, with a domination of the crossed projection. Shell-neurons in the white matter surrounding the LSO project to the inner hair cell region, with the uncrossed projection strongly dominating. The axons of MOC- and shell-neurons send collaterals into the ventral cochlear nucleus (VCN).

We found that many olivocochlear neurons survive unilateral cochleotomy. They were stained with the axonal tracer Fast Blue from an intact cochlea. This cochlea was removed one week later. Two months after cochleotomy, approximately two thirds of the LOC-neurons have disappeared. By contrast, no reduction was seen in the number of either MOC- or shell-neurons, showing that all these neurons survive cochlear lesion. These neurons may survive because they still have intact collaterals to regions other than the destroyed cochlea.

Following cochleotomy, reorganisation processes in the VCN were shown by emergence of fibers and boutons positive for the growth associated protein GAP-43. *In Situ*-hybridisation showed strong upregulation of GAP-43 mRNA in LOC-, MOC- and shell-like neurons, suggesting that olivocochlear neurons may be responsible for the sprouting activity in VCN. In order to prove this case, stereotactic injections of kainic acid into the SOC were done to destroy cell bodies residing there. If neurons in the SOC are responsible for the sprouting in VCN then removal of SOC before cochlear lesion must reduce this growth activity.

In rats where the LSO and the shell-region ipsilateral to the cochlear lesion were destroyed, no reduction in cochleotomy-induced GAP-43-immunoreactivity was seen. However, in animals where the SOC contralateral to the cochlear lesion was completely removed, i.e. including the VNTB, GAP-43-immunoreactivity in VCN was significantly reduced compared to animals that had a cochleotomy with an intact SOC (46,3%, $p=0,0212$). This supports the suspicion that the sprouting of neuronal structures in VCN is due to neurons whose cell bodies reside in the SOC contralateral to the cochlear lesion. However, as the sprouting activity does not fully disappear, these neurons cannot be the only source for cochleotomy-induced GAP-43 immunoreactivity in VCN.

Our findings make MOC-neurons, but not LOC- or shell-neurons, likely candidates for the cochleotomy-induced sprouting activity in VCN.

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951 Training modulates learning and performance levels of sensorimotor behaviour following dopaminergic grafts

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The current study focuses on the influence of motor training on lesion-induced behavioural impairment and graft-dependent functional recovery in a rat model of Parkinson's Disease. It compares motor training of different intensities and time-courses with the degree of functional recovery firstly after 6-Hydroxydopamine-lesion, and secondly following transplantation of nigral grafts into the lesioned striatum.

We applied a four-phase training-program, including both simple (stepping, sidefalling, tablelift) and complex (paw reaching) sensorimotor tests during all phases. Phase one started prior to a unilateral, complete lesion of the nigrostriatal pathway through 6-Hydroxydopamine injection into the medial forebrain bundle. Phase two followed in two training sessions post-lesion. Phase three was applied directly after transplantation of dopaminergic grafts from the fetal (embryonic day 14) ventral mesencephalon, and phase four includes a time delay following transplantation.

The results of post-lesion training in the paw-reaching test revealed a significantly better performance in complex motor tasks in pre-lesion trained rats both on the lesioned and on the healthy side in comparison to those animals not trained prior to the lesion. Furthermore, in early post-transplantation testing, a faster, graft-induced recovery was seen in the paw-reaching test in grafted and pre-lesion trained animals compared with post-lesion trained and non-trained rats and sham controls. In simple motor tests, no difference was observed between the groups.

This indicates that the degree of functional graft-induced restoration is significantly modulated by learning and training behaviour.

952 Transplantation of differentiated murine embryonic stem cells in a 6-hydroxydopamine rat model of Parkinson's disease.

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Parkinson's disease (PD) is a neurodegenerative disorder characterised by a gradual loss of dopaminergic neurons in the nigrostriatal pathway. Transplantation of foetal mesencephalic cells has been intensively studied in rat models of PD. However, technical difficulties in obtaining sufficient donor foetal brain tissue (for PD patients) have limited the application of this therapy and shifted the focus towards the use of embryonic stem (ES) cells. Recently, Björklund et al. (2002) demonstrated that transplantation of undif-

ferentiated ES cells into 6-hydroxydopamine (6-OH DA)-lesioned rats results in a proliferation of the ES cells into fully differentiated, functional dopaminergic neurons. However, 20% of the rats developed teratoma-like tumours, a problem which might be avoided by transplanting more differentiated cells. The aims of this study were to evaluate the integration and risk of tumourigenesis by transplanting differentiated mouse ES cells into adult rats with a unilateral 6-OH DA-induced lesion of the nigrostriatal pathway and to investigate methods to enhance the in-vivo survival rate of the transplanted cells. Mouse ES cells were differentiated on a PA6-feeder for 14 days. 25 – 30% of the obtained neurons were positive for tyrosine hydroxylase (TH). The cells were labeled with PKH26, a lipophilic fluorescent dye that binds irreversibly to cell membranes, and transplanted as a suspension into the striatum of 6-OH DA-lesioned rats. The rats received either daily injections of cyclosporin A (10mg/kg) or no immunosuppressive treatment. Five weeks after transplantation, transplanted cell survival and host responses were determined immunohistochemically using the following antibodies: TH, glial fibrillary acidic protein (GFAP)and ED1 – a marker for activated microglia/macrophages. PKH26 fluorescence showed labeled cells in all the transplanted animals. In non-immunosuppressed animals, a few TH-immunoreactive cell clusters were detected. These were however surrounded by a thick astroglial wall and the cells did not migrate into the surrounding host tissue. In immunosuppressed animals, there was enhanced survival of the transplanted cells (increased cell counts) and individual neurons with processes migrating into the host striatum were observed. Other effects included an increase in the graft volume and reduced astrogliosis at the host-graft interface. There was no marked difference in the accumulation of ED1-positive microglia/macrophages between the immunosuppressed and non-immunosuppressed animals. In all the animals, there was no evidence of tumour formation. These data demonstrate that ES-cell derived differentiated dopaminergic neurons do survive and show structural integration in the host striatum. In addition cyclosporin A treatment is effective in enhancing the survival of intrastriatal xenografts.

Increased neurogenesis after experimental Streptococcus pneumoniae meningitis

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Objectives: Proliferation of neural progenitor cells is increased in response to several modes of brain injury. In this study, proliferation of progenitor cells after experimental S. pneumoniae meningitis and expression of neuronal marker proteins suggesting differentiation into mature neurons was investigated.

Methods: Male C57BL/6 mice (n=35) were infected by injection of 4 log CFU of S. pneumoniae into the right forebrain. Controls (n=30) received an injection of saline. 24h later antibiotic therapy was initiated with ceftriaxone 100 mg/kg twice daily over 5 days. On day 2, 6, 10 and 16 after infection 4 respective groups of mice (6 infected animals, 6 controls) received 5 intraperitoneal injections of bromodeoxyuridine (BrdU) 50 mg/kg at 3-hourly intervals and were sacrificed 18h later. To assess long-term survival of BrdU-

labeled cells, 6 infected animals and 6 controls were treated with BrdU (50mg/kg) twice daily from day 7 until day 10 after infection and were killed 4 weeks thereafter.

Results: All mice infected with S. pneumoniae developed meningitis. 5 mice died during the acute phase of meningitis and were excluded from the analysis. The other animals fully recovered from the infection. Repeated tight-rope tests revealed a higher impairment of physical activity in meningitic mice compared to non-infected controls ($p=0.001$). At 6 days after infection, the density of BrdU-labeled progenitor cells in the dentate gyrus of the hippocampal formation was higher in the meningitis group than in controls, [median (25th/75th percentile): 22.2/mm^2 (16.7/26.4) vs. 8.7/mm^2 (5.8/9.5); $p=0.004$]. At 2 and 10 days after infection differences almost reached statistical significance ($p=0.06$). Survival of newborn cells 4 weeks after the last BrdU injection was higher after meningitis than in controls as indicated by double-labeling for BrdU and the neuronal marker MAP-2 [33.8/mm^2 (3/80.8) vs. 0.7/mm^2 (0.4/2.8); $p=0.005$]. Approximately 60% of BrdU-labeled cells differentiated into neurons as indicated by MAP-2 labeling. Cells stained also positive for the neuronal markers TUC-4 and β-tubulin.

Conclusion: In a mouse model of S. pneumoniae meningitis, proliferation of neural progenitor cells was enhanced in the subgranular layer of the dentate gyrus. Cells differentiated into mature neurons as indicated by expression of TUC-4, MAP-2 and β-tubulin. Endogenous repair mechanisms may limit the consequences of neuronal destruction after meningitis.

954 Endothelin-1 modulates astrocytic protein content and morphology by inhibition of gap junctional permeability

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Astrocytic endothelin (ET) receptors modulate calcium signaling and the astrocytic gap junctional network. The non-selective ET receptor ligand ET-1 inhibits gap junction permeability, an effect which can be blocked by tolbutamide. This mechanism may play a role in pathophysiological conditions such as ischemic stroke, characterized by elevated tissue ET-1 levels and hypertrophically appearing reactive astrocytes. Thus, we wanted to explore the effect of ET-1 on cellular protein content and morphology in cultured rat astrocytes. Confluent once passaged rat astrocyte cultures were used under serum-free conditions. Protein content was measured using the Lowry method. Gap junction permeability was determined by the scrape-loading/dye transfer technique.

ET-1 prevented the decrease in astrocytic protein content observed in controls. The effect of ET-1 on cellular protein content was most pronounced in cultures seeded at high density but attenuated in ETB-receptor deficient (sl/sl) astrocytes. The ET-1-induced increase in astrocytic protein content could be blocked by the non-selective ET-

antagonist LU 302872 (10μM) as well as the protein synthesis inhibitor cycloheximide (10μM) while it was antagonized by the ATP-sensitive K^+ channel blocker tolbutamide (400μM). Tolbutamide also antagonized the ET-1 induced reduction of gap junction permeability and reversed the morphological changes observed in astrocytes upon ET-1 treatment. Cytosine arabinoside (10μM), a DNA synthesis blocker, inhibited the ET-1-induced BrdU uptake without affecting the ET-1-induced increase in astrocytic protein content.

To conclude, ET-1 induces an increase in astrocytic protein content as well as changes in astrocyte morphology *in vitro*. This hypertrophic response involves uncoupling of the astrocytic gap-junctional network and is not dependent on DNA synthesis. Since ET-1 has been described to protect astrocytes from hypoxic/ischemic injury *in vitro* and the absence of functional ETB receptors led to an augmented hypoxic/ischemic brain damage *in vivo*, these protective effects of ET-1 might also involve a modulation of the astrocytic gap junctional network. Moreover, therapeutic exploitation of the endothelin receptor ligands may provide novel means for neuroprotection via modulation of astrocytic gap junctions.

Long-term behavioral, morphological and molecular follow-up after discrete cortical lesion in mice

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BACKGROUND: In order to test the hypothesis that traumatic brain injury can induce long-lasting behavioral changes we have established a discrete cortical freezing-injury model in young (4 weeks old) C57BL6 mice and studied the effects of the lesion in a long-term follow-up (3,6 and 9 months). We have characterized the model in terms of morphological and functional changes including histology, gene expression, blood-brain-barrier function, behavior and volumetric changes in brain-MRI and evaluated the potential neuroprotective effect of the hematopoietic growth factor erythropoietin (EPO).

METHOD: After incision of the skin the bregma was exposed and a brass cylinder cooled with liquid nitrogen was applied directly to the skull posterolateral (1.5mm/1.5mm) to the bregma in the right parietal hemisphere for 60 seconds. As a control we used sham-operated animals that received the same treatment without cooling the cylinder. The behavioral testing at 3,6 and 9 months included: Elevated-Plus-Maze, Open-field, Rotarod, Hole-Board, Morris-Water-Maze and Fear-Conditioning.

RESULTS: Imaging and histology showed a standardized and highly reproducible cortical lesion in all freeze-injured animals. The lesion reached its maximal volume 24h after injury, at which time-point the blood-brain barrier leakage was also maximal. The lesion

was detectable by MRI throughout the 9-month follow-up period. There were no significant changes in hippocampal or lateral ventricular volume between the groups.

Behavioral testing revealed selective changes in exploration- and fear-related behavior 3 months after the lesion, while deficits in learning- and motor-functions were seen delayed at 9 months after the lesion. Three months after the lesion we observed an increase in percentage of open arm entries for the lesion group (72.49±3.75% vs 58.11±3.69% in controls, p=0.01, n=10 for each group) in the Elevated-Plus-Maze. This behavior was accompanied by an altered exploratory behavior. The exploration- and fear-related behavioral alterations 3 months after the lesion could be reversed by intraperitoneal application of EPO (5 U/g every other day for 14 days, starting immediately after application of the freezing lesion). Neither sham-operation nor treatment with EPO alone induced behavioral changes.

SUMMARY & CONCLUSION: The cortical freeze-injury model in mice has been established and characterized in terms of behavior, morphology and changes in gene-expression. We characterized the model in a long-term follow-up after 3, 6 and 9 months. The selective and distinct behavioral alterations can be treated successfully with a neuroprotective intervention.

956 Expression of growth-related genes predicts different regenerative capacities of neurons with a spinal axon and indicates plasticity of intraspinal neurons in adult zebrafish

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The spinal cord of adult zebrafish is considered to be a generally favorable environment for axon regrowth after a lesion. However, we find strong differences in the regenerative capacity between different neuronal populations that are axotomized by the same spinal lesion. In brain nuclei of high regenerative capacity, a mean proportion of 36% of the neurons regrow their descending axons and 86% of the axotomized neurons express high mRNA levels of the growth-related molecules L1.1 and GAP-43, which are markers of a regenerative response. In brain nuclei of low regenerative capacity, 13% of the neurons regrow their descending axons and 50% show high expression levels of growth-related molecules, indicating that gene expression correlates with regenerative success. Surprisingly, retrograde tracing from the brainstem/spinal cord transition zone revealed that only 2 to 4% of the spinal neurons with ascending axons regenerate their axons after the same spinal lesion. Combining tracing with *in situ* hybridization shows that only 24% of the axotomized neurons in the spinal cord express high mRNA levels of GAP-43 and L1.1. The distance of the lesion site from the somata of spinal neurons did not influence axon regrowth or gene expression. Thus, spinal neurons with ascending axons show an intrinsically weak regenerative response in the growth-permissive spinal environment, whereas descending axons show a good regenerative response, correlated with the upregulation of growth-associated genes. Intraspinal plasticity may also contribute to recovery, as we have found a transient increase in expression of growth-related molecules in a substantial population of neurons that have not been axotomized in the lesioned spinal cord. This may indicate significant plasticity in spinal-

intrinsic circuitry after a lesion. We suggest that the high degree of locomotor recovery observed after spinal lesion in adult zebrafish is influenced less by recovery of ascending pathways, but more by regrowth of descending tracts and rearrangement of intraspinal circuitry.

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Neuronal regeneration in the ventral nerve cord of the locust 957

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Whereas regeneration of peripheral neurons after nerve injury is quite common throughout the animal kingdom, the central nervous system (CNS) appears to be less capable of regeneration, at least in humans. One of the main reasons for the lack of ability to regenerate is the negative influence of glia cells. However, neuronal regeneration has been observed in a variety of invertebrate species not only in the PNS (e.g. Lakes and Kalmring, 1990, J. Neurobiol. 22:169-181; Stern et al., 1997, J. Neurobiol. 33: 439-458) but also in the CNS (e.g. Bittner, 1991, TINS 14:188-192; v Bernardi and Muller, 1995, J. Neurobiol. 27:353-366, Spira et al., 1987, J. exp. Biol. 132:111-131).

It is generally assumed that the same processes that control the formation of the nervous system during embryonic development are involved in neuronal repair as well. Unfortunately, there are only few preparations on which this can be directly tested. Among the insects, neuronal development has been most intensively and thoroughly studied in *Drosophila* and the Locust (review: Goodman, 1996, Annu Rev Neurosci 19:341-377). Since regeneration experiments are not easy in *Drosophila*, mainly due to its small size, we choose the Locust.

As a first step, we used an immunocytochemical approach to monitor regeneration in the central nervous system after crushing one neck connective in the third larval instar. A peptide neurotransmitter, like proctolin (Ude and Eckert, 1988, Cell Tissue Res 254:197-202), is a useful tracer to reveal a small set of identified neurons in the ventral nerve cord. The neck connective contains two prominent proctolin-immunoreactive axons that originate in the thoracic ganglia and project into the brain, arborising in a distinct pattern *en route* in the subesophageal ganglion (Keshishian and O'Shea, 1985, J. Neurosci. 5:992-1004). The nervous systems were dissected and immunostained for proctolin on various days post crush.

Within the connective, the severed axons formed a retraction bulb and started sprouting within the first day post crush. After three days, the axons had begun to grow into the lesion site. Six days post crush, most neurons had grown past the lesion site. Most of the neurites had reached the subesophageal ganglion by nine days post crush, and started to form an arborisation. Within the following week, this arborisation matured and the neurons reinnervated their target area, however without reassuming their original morphologies. Their aberrant morphologies clearly characterised them as regenerated neurons, thus excluding the possibility of an incomplete crush in those preparations.

We conclude that post embryonic neuronal regeneration is possible in the Locust ventral nerve cord, which enables us now to study the underlying mechanisms in this animal and to compare them to the well described embryonic development.

We are grateful to Manfred Eckert for providing the anti-proctolin antiserum.

958 Analysis of the endogenous FGF-2 system with regard to its role after peripheral nerve lesion

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Basic fibroblast growth factors (FGF-2) is involved in several cellular processes of the nervous system, including neurite outgrowth and survival of injured neurons.

FGF-2 and its high affinity receptors (FGFR)1-3 are expressed in the peripheral nervous system and were up-regulated at the lesion site and in the dorsal root ganglia (DRG) following nerve injury.

To study the physiological role of the endogenous FGF-2 system, we analyzed the sciatic nerve and spinal ganglia L4, L5 with a morphometric system after nerve crush (regeneration model) and axotomy (degeneration model) in mutant mice.

1. Regeneration. In uninjured sciatic nerves no difference concerning number and diameter of myelinated axons were detected between FGF-2 knock out mice and wild type animals (129P2xBlackSwiss). One week after nerve crush, FGF-2 deleted mice displayed a significant lower reduction of myelinated axons (0,5 mm distal: -59%) compared to wild type (-93%).

2. Degeneration. In intact DRGs, the number of sensory neurons was unchanged in FGFR3 ko mice compared with wild type mice (C57BL/6). One week after axotomy, 25% of the DRG neurons was lost in wild type animals. In contrast, FGFR3 deleted mice showed no loss of sensory neurons.

We propose that FGF-2 and its receptor FGFR3 are critical for axonal regeneration and axotomy-induced cell death.

FGF-2 isoforms in postmitotic sympathetic neurons: Synthesis, nuclear transport and involvement in karyokinesis

959

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Alternative initiation codons lead to synthesis of low (18kDa) and high (21 or 23kDa) molecular weight isoforms of the multifunctional cytokine basic fibroblast growth factor (FGF-2) which are all expressed in the peripheral nervous system. The aim of the present study was to analyze the localization and possible effects of FGF-2 isoforms in cultured sympathetic neurons after exogenous treatment and endogenous overexpression, respectively. Dissociated sympathetic neurons derived from postnatal day 6 rat superior cervical ganglia reveal weak nuclear FGF-2 levels immediately after plating. At later stages, FGF-2 immunoreactivity increases in the nucleus and in the perinuclear region corresponding to the Golgi apparatus. Introducing plasmids encoding the FGF-2 isoforms fused to fluorescent proteins into neurons reveals nuclear targeting of FGF-18kDa and FGF-23kDa with prominent accumulation in the nucleolus. Addition of FGF-2 isoforms to sympathetic neurons, which exhibit membrane bound as well as nuclear FGF receptor 1, has no effect on survival or axon outgrowth in the presence or absence of nerve growth factor. Overexpression of FGF-23kDa in dissociated neurons or in organotypic ganglion slice cultures, however, results in a significant increase in the proportion of binucleated neurons when compared to overexpression of a fluorescent control protein in the neuronal nucleus. The N-terminal part of FGF-23kDa, which excludes the 18kDa sequence, reveals a nuclear distribution also and is sufficient for this effect to occur. FGF-18kDa does not have an influence on either karyo- or cytokinesis. Therefore, while FGF-2 is a mitogen for neuroblasts and a variety of non-neuronal cell types, treatment with FGF-2 does not induce mitosis of fully differentiated sympathetic neurons but a subpopulation of neurons overexpressing the high molecular FGF-2 isoform are forced to undergo amitotic karyokinesis and show signs of cytokinesis before degeneration.

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960 Axotomy induced reversed microtubules polarity leads to the formation of a vesicles trap and the extension of a growth cone's lamellipodium

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The transformation of a stable axonal segment into a motile growth cone is a critical step in the regeneration of amputated axons. A striking observation made in earlier studies from our laboratory, is that down stream, in the cascade leading to growth cone formation, anterogradely transported vesicles accumulate at a defined compartment some 100-150mm proximal to the tip of the cut axonal segment. This compartment forms the GC's center.

Here we began to explore the mechanisms by which vesicles are trapped at this specific location. To that end, we expressed EGFP-EB3 in cultured Aplysia neurons. EGFP-EB3 binds in stretches to the plus end of microtubules, moves with the growing MT's tips forming a comet tail like structure and thereby allows to image the dynamics and polarity of MTs.

We found that axotomy leads to restructuring and reorientation of the MTs polarity at the cut axonal end. 100-150mm from the cut axonal tip where the GC center is formed and vesicles are trapped, the MTs reorient such that plus ends point towards a common center- the trap. Proximally the trap is formed by MTs that maintain their original polarity. The distal boundary of the trap is formed by MTs with reversed polarity. Distal to that, following a short axoplasmic gap the MTs point their plus ends anterogradely.

We propose that the vesicles trap is formed by the reorientation of the MTs polarity thus directing molecular motors to deliver vesicles into the trap.

961 Reversible internalization of voltage gated channels accompany brefeldin A-induced structural remodeling of cultured *Aplysia* neurons

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Eucaryotic cells constitutively internalize the plasma membrane. Nevertheless, the morphology and membrane properties of the cells are maintained constant by compensatory constitutive exocytosis. Recently, we reported that the neurites and axons of cultured Aplysia neurons are internalized within 24-48 hr of 10 mg/ml brefeldin A application (Prager-Khoutorsky and Spira 2000, Neuroscience Letters supplement 55, S43). When BFA is washed away, the Golgi apparatus reassembles, and the neuron extends new neurites.

Here we report that the passive and active membrane properties of the neurons undergo alterations during these restructuring processes.

Control experiments revealed that both the cell body and axons are excitable and that the action potentials are not blocked by BFA.

We found, that for as long as the axons are not totally internalized (in BFA) depolarization of the neuron evokes action potentials. Nevertheless, when the axons are internalized, depolarization of the formally excitable cell body fails to generate action potentials. We thus conclude that the cell body looses it excitability during the axon's membrane internalization. Cytochalasin B inhibits the effects of BFA. Under these conditions the cell's input resistance and excitability are not altered.

When BFA is washed and the naked cell body begins to extend growth cones, depolarization generates normal action potentials. These observations suggest that the newly formed vesicles carry to the plasma membrane the normal repertoire of voltage-gated channels to generate action potentials. We conclude that regulation of the balance between constitutive exocytosis and endocytosis leads to remodeling of the morphology and the biophysical properties of adult neurons.

Critical calpain-dependent ultrastructural alterations underlie the transformation of an axonal segment into a growth cone after axotomy of cultured *Aplysia* neurons **962**

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The transformation of a stable axonal segment into a motile growth cone is a critical step in the regeneration of amputated axons. In earlier studies we found that axotomy of cultured Aplysia neurons leads to a transient and local elevation of the free intracellular Ca^{2+} concentration, calpain activation, localized proteolysis of the submembrane spectrin and growth cone formation. Inhibition of calpain by calpeptin prior to axotomy inhibits growth cone formation.

Here we investigated the mechanisms by which calpain activation participates in the transformation of an axonal segment into a growth cone. To that end we compared the ultrastructural alterations induced by axotomy of cultured Aplysia neurons performed under control conditions and in the presence of calpeptin. We identified critical calpain-dependent cytoarchitectural alterations that underlie the formation of a growth cone after axotomy.

Calpain-dependent processes lead to restructuring of the neurofilaments and microtubules to form a specialized cytoskeletal compartment 50-15-mm proximal to the tip of the transected axon. This compartment "traps" transported vesicles and serves as a locus for microtubule polymerization. As a result, a dense pool of vesicles accumulates in close proximity to a segment of the plasma membrane along which the spectrin membrane skeleton was proteolyzed by calpain. We propose that this facilitates the fusion of vesicles with the plasma membrane, promoting the extension of the growth cone's lamelli-

podium. The growth process is further supported by the radial polymerization of microtubules from the growth cone's center.

963 On line confocal imaging of processes underlying the dedifferentiation of an axonal segment into a motile growth cone after axotomy

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Regeneration of neuron after mechanical injury requires structural and functional dedifferentiation of the cut axonal end into a motile growth cone (GC). Here we analyzed the events leading to the formation of a growth cone (GC) and examined the mechanisms that set the dedifferentiation process into motion.

To that end we fluorescently labeled actin by chimeric EGFP-actin, microtubules (MTs) by tetramethylrhodamine conjugated tubulin, and vesicles by the styril dye RH-237, in primary cultures of identified Aplysia neurons.

Confocal imaging revealed that: (a) axotomy triggers a wave of MTs depolymerization. The depolymerizing wave stops at a point 100-150 mm from the cut axonal end, forming a transition zone (TZ) between the depolymerized segment and a proximal segment in which the MTs maintain normal structure. (b) In parallel, actin reach adhesion plaques disappear only to reform minutes later. (c) Anterogradlly transported vesicles are trapped within the TZ. Thereafter, (d) actin bundles assemble along the axons perimeters surrounding the vesicles trap. Once the above-described structure is formed an actin reach GC lamellipodium, supported by radial polymerization of MTs, begins to extend.

Axotomy in the presence of nacodasol prevents the restructuring of the depolymeryzed MTs and the trapping of vesicles, but does not affect the initial extension of a GC's lamellipodium. In jusplakinolid the MTs based vesicles trap is formed, but the extension of a GC's lamellipodium is blocked. Calpeptin totally inhibits the restructuring of the axon (Oren et al., this meeting).

We conclude that a common calpeptin sensitive event triggers the dedifferentiation process.

How Likely is Recovery after a Stroke? – Implications from Descriptive Movement Analysis after Focal Cerebral Ischemia in Adult Rats 964

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Previous experiments indicated that small cortical lesions lead to initial behavioral impairments that substantially improve during the early period after the insult. Nevertheless, it has not been shown what constitutes this improvement, i.e. whether behavioral changes represent a restoration of function (recovery) or the development of new strategies different from the original function (compensation). The differentiation between recovery and compensation requires a thorough and detailed behavioral analysis that can be accomplished by the analysis of skillful forelimb movements. The purpose of this series of studies is to investigate whether early motor improvements after focal cerebral ischemia correspond to recovery or compensation. Adult Wistar rats were pretrained in a skilled reaching task to grasp and retrieve food pellets and then received a unilateral photothrombosis in the forelimb area of the motor cortex. Animals were continuously tested in the reaching task until no more improvements in success rates were measured (three weeks after lesion). The end point measures revealed that success rates of retrieving food pellets returned to pre-lesion levels, however, qualitative analysis of reaching movements indicated chronic and severe impairments in forelimb movements. These data indicate that animals acquired alternative movement strategies to successfully obtain food pellets and suggests that skilled reaching tasks are a sensitive tool to analyze behavioral plasticity and subtle lesion effects. Corresponding histological data to evaluate structural rearrangements are currently under investigation.

Postlesional vestibular reorganization alters the spatial tuning of the frogs translational VOR 965

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The consequences of postlesional synaptic plasticity for the spatial tuning of the translational vestibulo-ocular reflex (tVOR) were studied in frogs two months after a selective section of the ramus anterior of the VIIIth nerve. This nerve section eliminates the inputs from the utricular, the anterior vertical and the horizontal semicircular canal, whereas inputs from the posterior vertical canal and the lagena (a vertical macula organ) remain intact. From previous studies (Goto, Straka and Dieringer; 2002) it is known that second order vestibular neurons, disfacilitated due to afferent nerve section, become receptive to additional excitatory inputs, preferentially from intact vestibular nerve afferent fibers. The spatial tuning encoded by the output of these second order vestibular neurons therefore appeared to change in an undesirable way.

Decerebrated and immobilized frogs were accelerated in darkness sinusoidally (typically at 0.5 Hz ± 1.8 g) on a sled either horizontally, vertically or in any inclination between these two extremes. Abducens nerve responses were recorded either on the intact or on the lesioned side. Responses were filtered and the calculated instantaneous discharge rates were smoothed, averaged over several cycles and fitted with a sine wave to characterize depth of modulation and phase.

The abducens nerve response magnitude evoked by horizontal sinusoidal acceleration depended on the static head position with respect to the direction of sled motion. The directions of maximal responses for the abducens nerve on the intact side were more diverse and tended to point more laterally compared to intact animals. The angle between the body length axis and the direction of the sled that evoked maximal responses was in intact frogs 70° ± 8° (n=11) but 94° ± 18° (n=9) in chronic RA-frogs. In addition, vertical sinusoidal acceleration evoked abducens nerve responses in chronic RA-frogs. Such a response component could not be seen in intact animals. The abducens nerve responses on the operated side remained unaltered as compared to intact frogs. The postlesional reorganization of the tVOR therefore resulted in asymmetric alterations with the consequence that conjugate eye movements became impaired. Thus, the reorganization appears to have given more priority to the survival of disfacilitated second order vestibular neurons than to the recovery of functionally adequate reflex behavior.

966 Studies of the regenerating marmoset (*Callithrix jacchus*) retina proteome

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Purpose: Axons of the central nervous system (CNS) display a limited regeneration. In order to examine the regenerative abilities of mature retinal ganglion cells (RGCs), an organ culture system of total monkey retina was established. The two goals of this study were to quantify and qualify the aspects of growth and to analyse the protein expression of the regenerating retina.

Methods: Fresh retinas obtained from monkey cadavers of various ages were explanted with the ganglion cell layer (regenerative group) facing the laminin-1 (α_1, β_1, γ_1) coated surface. Explants with no contact to laminin-1 and non-explanted retinas served as controls. After 3 or 5 days in culture, fibre numbers were estimated and explants were analysed by immunohistochemistry and proteomic investigations for regeneration- and growth-associated proteins.

Results: Massive regeneration of axons occurred in the regenerative group with the RGCs being the only cell type contributing to axonal regeneration. The propensity to regenerate axons decreased with age. Immunohistochemistry revealed positive staining for growth-associated proteins like GAP-43 and integrin $\alpha_5\beta_1$. Protein homogenates of the explants could be separated by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and appeared in a reproducible pattern of about 500 to 1000 spots using colloidal coomassie blue or silver staining, respectively. Ongoing analysis is devoted to

identifying regularly and differentially expressed spots for mapping and other procedures. However, encouraging data are provided by the total mapping and certain proteins like α-crystallin seem to be discernible and selectively upregulated after axonal growth.

Conclusion: Primate retinal ganglion neurons unequivocally possess a high regenerative potential. Unravelling of the mechanisms of initiation and maintenance of this growth will be useful in understanding how to use this potential in brain repair.

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Pattern of GABA-immunoreactive neural structures in the original and regenerated ventral nerve cord ganglia of the earthworm, *Eisenia fetida* 967

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Several earthworm species have enormous potential to regenerate considerable portions of the body loss by injury. The presence of the neural structures is required for reconstruction to proceed. The damaged ganglion could have dual role in regeneration: (1) it mediates the formation of regeneration blastema and differentiation of multipotent neoblasts by liberation neurohormones and/or certain neurotransmitters, futher (2) it reconstitutes its losted part by reorganization of the neural structures. However, in these days it is not clear whether morphogenesis or morphallaxis play key role in the neural regeneration.

To clarify this question caudal regeneration of *Eisenia fetida* has been investigated on the third postoperative week when the 8-15 segments reconstituted. By application of whole mount immunocytochemistry we revealed the three-dimensional structure of the GABA-immunoreactive neurons (both somata and processes) in the original, severed and reconstituted ganglia, further in newly formed ones of regenerated segments. Pattern of GABA-IR structures proved to be identical in the original and newly formed ganglia: we found no discrepancy either in cell number or in anatomy of neural processes. In contrast the reconstituted ganglion showed abnormal gross anatomy and pattern of GABA-IR structures, as well. Identifying the border between the old and new ganglion parts no change in the anatomy of GABA-IR somata was found in the old part, however, marked axonal sprouting was observed. The reconstituted part has extranumerous segmental nerves up to 7 pairs further irregular pattern of GABA-IR somata has been seen in which only a few cell groups seem to be identical with GABA-IR neurons of the old ganglia. These results suggest that severed stumps of CNS axons regenerate rapidly in *E. fetida* but reconstituted part of the ganglion could form from the cells of the regeneration blastema.

968 The expression of the cyclin dependent kinase inhibitors p16 and p18 in the gerbil Organ of Corti.

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It has recently been shown that the cyclin dependent kinase inhibitor (cdk) $p27^{Kip1}$ is important for the development of the mammalian Organ of Corti. The expression of p27 in the supporting cells follows the final cell divisions in the Organ of Corti and continues permanently preventing supporting cells in the mammalian inner ear from reentering the cell cycle. The cdks form two families: the Cip/Kip group including p21, p27 and p57 and the INK4 group including p16, p15 and p18. While p27 knock out mice show increased numbers of hair cells and supporting cells, they retain a recognizable Organ of Corti indicating that some compensatory factors may limit uncontrolled cell proliferation. We have begun to search for the expression of other cdks in the gerbil Organ of Corti to identify such potentially interacting and compensating factors.

The expression of cdks was studied immunohistochemically and commercial antibodies were tested on paraffin sections of gerbil spleen and intestine to demonstrate that they recognized the gerbil cdks. Presently we have identified antibodies against human p27, mouse p16 and mouse p18 peptides (Santa Cruz) that react with gerbil tissue. To investigate specific expression of the cdk in the inner ear, cross sections of the decalcified and paraffin embedded Organ of Corti were incubated with the respective antibodies and binding was visualized using a biotinylated secondary antibody with a subsequent DAB reaction.

With the p27 antibody we obtained prominent staining in Deiter, Hensen and Pillar cells, absence of staining in outer hair cells and some diffuse staining in inner hair cells. These data are consistent with previous reports of p27 expression in the mouse cochlea. With the p18 antibody we obtained prominent staining in the Deiter and Hensen cells low or absent staining in the Pillar cells, no staining of outer hair cells and absent or weak staining of inner hair cells. The p16 antibody showed a highly specific staining of Deiter and Pillar cells, and did not stain Hensen cells or hair cells.

The observation that cdks are differentially expressed in the different types of supporting cells in the gerbil Organ of Corti are relevant for understanding the mechanisms that prevent hair cell regeneration in the mammalian cochlea.

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Partial Recovery of Conditioned Taste Aversion after Stem Cell Transplantation in Insular Cortex Lesioned Rats. 969

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The insular cortex (IC) is involved in the mnemonic gustatory representation and is an important region for the acquisition, consolidation and retrieval of the conditioned taste aversion task (CTA). Previously, it has been demonstrated that impairment of CTA learning due to IC lesions can be restored with homotopic fetal brain grafts. The goal of this study is to investigate the potential of adult stem cells of the subventricular zone (SVZ-SCs) to promote the behavioral recovery after IC lesions, which is measured as CTA.

Adult male Wistar rats were stereotaxically lesioned in the IC of the brain. Five days after the lesion, control and lesioned rats were trained and tested for CTA. Two extinction trials were performed after the CTA test. Adult SVZ-SCs were pre-labeled *in vitro* and transplanted into the lesion area. Two months after the transplantation, all groups (intact, lesioned and transplanted rats, n=4 for each group) were again trained and tested for CTA. One-way ANOVA, and post-hoc multiple comparisons were performed between all groups for the fluid intake of the test day. At the end of the behavioral experiment, all animals were prepared for histological analysis.

Our results indicate, that prior to the transplantation, IC-lesioned rats showed a disrupted taste aversion when compared with intact controls ($p<.05$). After the graft, the SVZ-SC's transplanted rats showed an aversion of approximately 70% of the intact group which was significantly better than before transplantation, while the lesioned rats showed no aversion ($p<0.05$). Histological analysis indicates an extensive migration and integration of the transplanted SVZ-SCs into the host tissue. Our results show, that adult SVZ-SCs are able to integrate into the host tissue and that they can promote a partial functional recovery of IC-lesioned rats.

Treatment of auditory phantom perception (tinnitus) with neuronavigated repetitive transcranial magnetic stimulation (rTMS) – a pilot study 970

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OBJECTIVES

The pathophysiological mechanisms of idiopathic tinnitus remain unclear. Recent studies demonstrated focal brain activation in the auditory cortex of patients suffering from

chronic tinnitus. Low frequency repetitive transcranial magnetic stimulation (rTMS) is able to reduce cortical hyperexcitability.

METHODS

Patients suffering from severe chronic tinnitus underwent a [18F]deoxyglucose- PET (positron emission tomography) and MRI (magnetic resonance imaging) measurement. Fusioning of the individual PET scan with the structural MRI-scan (T1, MPRAGE) allowed to exactly identify the area of increased metabolic activity in the auditory cortex, which was selected as the target point for rTMS. In this context, a neuronavigational system adapted for TMS positioning enabled to monitore the exact position of the figure 8-shaped magnetic coil in relation to the target area. rTMS (110% motor threshold; 1 Hz; 2000 stimuli/ day over 5 days) was performed using a placebo controlled cross-over design. Patients were blind regarding the stimulus condition. For the sham stimulation a specific sham-coil system was used. Treatment outcome was assessed with a specific tinnitus questionnaire (Goebel and Hiller)

RESULTS

Up to date, 10 patients were included. In 9 of 10 patients we could localize an increased metabolic activation in the upper dorsal part of the left superior temporal gyrus. After 5 days of verum rTMS a remarkable improvement of the tinnitus score was found. This effect could not be seen after sham stimulation.

CONCLUSION

These preliminary results demonstrate that neuronavigated rTMS offers new possibilities in the understanding and treatment of chronic tinnitus.

971 Neurofascin interactions in sensory neurons

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A highly complex and specific network of cell-cell and cell-extracellular matrix (ECM) interactions is needed during embryonic development to build up the nervous system. Immunoglobulin-like domains containing cell adhesion molecules (IgCAMs) form a part of this network, exhibiting a high degree of complexity in structure, functional interactions and expression pattern. They are involved in crucial developmental steps like neural migration, neurite induction, pathfinding and plasticity.

The IgCAM neurofascin is a glycosylated transmembrane molecule belonging to the L1 subfamily of the immunoglobulin superfamily of cell adhesion molecules (IgSF). Its complete cDNA sequence comprises 33 exons, ten of which are differentially spliced resulting in at least 50 splice variants. It has been shown that neurofascin is able to interact extracellularly with other molecules of the IgSF such as NrCAM, axonin-1 and F11. By the use of different combinations of alternatively spliced exons, binding behaviour of neurofascin to receptor axonin-1 can be modulated. The aim of this work was to determine neurofascin splice variants present in early (embryonic day 5 (E5)) and late

(embryonic day 9 (E9)) chick embryonic sensory dorsal root ganglia (DRG) neurons. Furthermore, receptor usage of sensory neurons on a neurofascin substrate was investigated.

Different splice variants were detected by PCR screening with exon specific primers using chick cDNA as template obtained from isolated DRGs of the respective embryonic stages. In early stages of chick embryonic development (E5), only one major early neurofascin isoform was detected in DRGs. Later in development (E9) three additional and distinct splice variants were identified. The late neurofascin isoforms differed from the early variant only in the proline alanine threonine rich domain (PAT) (exons 26 and 27) and the fifth fibronectin type III domain (exons 28, 29).

To investigate receptor usage of outgrowing chick sensory DRG neurons, antibody perturbation experiments using a neurofascin-Fc chimeric protein as substrate were performed. Neurofascin is a substrate for sensory neurons. Neurite outgrowth could be blocked completely by Fab fragments of antibodies specific for neurofascin. In the presence of Fab fragments of antibodies specific for axonin-1, no axonal outgrowth was detected. Axonal outgrowth was still possible with Fab fragments of antibodies specific to F11 present. No effects have been observed in the presence of Fab fragments of antibodies specific to NrCAM, which is in contrast to similar experiments performed with tectal neurons, where NrCAM specific Fab fragments completely inhibited neurite outgrowth on a neurofascin substrate.

Our results suggest that axonin-1 is used as the main receptor of sensory neurons on a neurofascin substrate. After axonin-1 antibody perturbation, DRG sensory axons could not extend neurites, whereas neurofascin/NrCAM or neurofascin/F11 interactions seemed to be of minor importance for the sensory axon to perform neurite outgrowth.

Organotypic co-cultures on MEA as a valuable tool to study the establishment of projection pathways in the CNS

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Injury to the adult mammalian central nervous system (CNS) caused by trauma, ischemia or neurodegenerative diseases results in serious and permanent functional deficits. Promoting repair and regrowth of neurons is an increasingly attractive approach to the treatment of neuronal injury. Evaluation of treatments that may lead to neural regeneration require model systems that closely reflect *in vivo* conditions and correlate long-term recordings of physiological activity with morphological changes. We here present an organotypic CNS culture model on micro electrode arrays (MEAs) which allows us to monitor long term effects of potential regeneration-promoting drugs on a functional level within the same culture for days to weeks.

Slices of entorhinal cortex (EC) and dentate gyrus (DG) were placed on MEAs and cultured up to 6 weeks. Connections between the two tissues were shown immunocyto-

chemically by neurofilament staining. The functionality and specificity of the newly formed connections was shown by electrical stimulation and parallel recording of correlated activity in the target tissue. About 80% of the connections *in vitro* were unidirectional, propagating signals only from the EC into the DC. Beginning at DIV (day *in vitro*) 2, formation of functional connections took place throughout the first week *in vitro*. In addition, high frequency stimulation (100 Hz) showed characteristics of monosynaptic signal transmission, indicating that the connections between the EC and DC formed *in vitro* reflect the perforant pathway projection between the two tissues *in vivo*. Pharmacological analysis identified glutamate receptors of the AMPA/kainate type as the main mediators of excitatory signal transmission in the DG.

To evaluate our co-culture of EC and DG as a model for neuronal regeneration, we tested the impact of C3d on the formation and specificity of neuronal projections. Cd3 is a synthetic agonist of the neural cell adhesion molecule NCAM which has been shown to promote neurite outgrowth in single cell cultures. Addition of C3d to the culture medium from DIV 0 significantly accelerated the establishment of connections without affecting their specificity. The acceleration could be blocked by antagonists to PLC-γ and c-fyn which are key mediators of the intracellular NCAM signaling pathways.

In conclusion, we have shown that our EC-DG co-culture on MEAs is a valid *in vitro* model for the perforant pathway *in vivo* and a useful tool to investigate long term pharmacological effects on synaptic activity and regenerative processes.

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973 Different Axonal and Glial Migration Velocities Determine the Tissue Engineering Concept of Artificial Nerve Guides

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The repair of traumatized nerves represents a considerable clinical challenge. A biodegradable, hollow nerve guide tube (Æ 1 mm) made of biocompatible polylactide-coglycolide was developed to bridge nerve lesions. The 100 µm thick, semipermeable wall of the nerve guide tube was micro-structured to allow nutrient diffusion but to prevent infiltration by scar forming, inhibitory fibroblasts. The untreated material surface was semipermissive for cell attachment. No oxygen plasma treatment was performed to increase the surface hydrophilicity, since the nerve guide stability was reduced by 50% after a 30 sec plasma treatment. Purified rat Schwann cells (SCs), fibroblasts, and dorsal root ganglia (DRG) were employed *in vitro* to imitate distinct *in vivo* situations. Encapsulation of SCs inside the tube showed that cells survived over a 7-days period. Co-cultivation with DRGs resulted in pronounced axonal outgrowth inside the nerve guide tube. Fibroblasts positioned on the outer tube surface were not able to penetrate the tube wall. Time lapse video recording and migration assays of synchronized SCs revealed that axons grew 8 times faster than Schwann cells migrate. In addition, SCs tended to randomly meander with changing velocities (4-70 µm/h), whereas axons

were extended in a directed fashion at a fairly constant speed (65 µm/h). Co-culture experiments with SCs and DRGs showed that axonal outgrowth was enhanced 15-fold, if neuritotrophic Schwann cells were pre-seeded onto polymer surfaces.

Our tissue engineering concept based on the combination of innovative materials and self-sustained biological components (i.e. SCs) aims at the development of an "intelligent neuroprosthesis" that first mediates regeneration and then disappears. To accelerate regeneration in this context, purified Schwann cells are best injected into synthetic nerve guides before implantation rather than to rely on slow SC immigration from nerve stumps.

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Ipsilateral motor pathway investigated by TMS and functional MRI in patients with recovered paralytic upper limb

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Introduction Studies in hemiparetic patients due to corticospinal tract injury strongly suggest an important role of ipsilateral cortex in functional reorganization of the motor system. Combined use of transcranial magnetic stimulation (TMS) and functional MRI (fMRI) would be powerful tools to investigate the mechanism of motor recovery and presence of ipsilateral pathway after brain injury. The purpose of this study is to delineate the ipsilateral motor pathways in patients who have been recovered from paralytic upper limb after brain injury using both fMRI and TMS.

Methods Eight criteria based patients with motor recovery after stroke (5 right hemiparesis, 3 left hemiparesis) were participated. The mean age was 49.2 years and the mean Edinburgh score before brain injury was + 99.4. The mean onset duration at the time of the fMRI experiment was 17.7 months (4 to 35 months). FMRI was performed on a 1.5T Siemens Vision scanner in all subjects. Repetitive flexion and extension of affected fingers at 1 Hz was used to activate motor network during fMRI. Twenty slices were acquired using single-shot EPI sequences (TR: 3840 msec, TE: 40 msec, Flip angle: 90°,

FOV: 220 mm, Matrix: 64 × 64, slice thickness: 6 mm). Each functional run consisted of 54 images representing 4 control-task pairs. The fMRI data was analyzed using SPM-99 software. Statistical parametric maps (SPM) were obtained and voxels were considered significant if their Z scores were significant at $p < .001$ uncorrected. TMS was performed to confirm the ipsilateral or contralateral motor pathway. The magnetic stimulation was applied to the scalp at 7 cm lateral to Cz using 90 mm round coil with 90% of maximal stimulation intensity. Recording was done on the bilateral abductor pollicis brevis muscles.

Results FMRI showed activation of bilateral primary sensory motor cortex (SM1) in 5 out of 8 patients (62.5%). Activation of only contralateral SM1 was seen in 2 patients. In remained 1 patient, contralateral SM1 and ipsilateral posterior parietal lobe was activated. Additional activation of the supplementary motor area was seen in 3 patients. TMS study confirmed ipsilateral motor pathway in 3 patients out of eight (32.5%). All of them showed ipsilateral SM1 activation in fMRI study. Five patients were confirmed to have only contralateral motor pathway in TMS study.

Conclusion About one third of patients with recovered paralytic upper limb following brain injury revealed to have activated ipsilateral motor pathways demonstrated by both fMRI and TMS studies. Subclinical mirror movements or changed threshold of ipsilateral motor pathways can be considered as the reasons of incongruence between these two modalities. (This study was supported by the Korean Brain Neuroinformatics Research Program and Korean Scientific and Engineering Foundation.)

975 Diverse pharmacological manipulations of nerve activity has a differential effect on activity-dependent genes in the cochlea and auditory cortex.

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Aberrant neuronal activity and plasticity changes are thought to be the basis for a variety of such neurological disorders as epilepsy, phantom pain and phantom noise (tinnitus). The activity-dependent nature of brain-derived neurotrophic factor (BDNF), c-fos and activity-regulated cytoskeletal gene (Arg 3.1) single them out as likely candidates in mediating synaptic plasticity changes. Using *in situ* hybridisation and semi-quantitative RT-PCR, we analysed the expression pattern of these genes in the adult Wistar rat cochlea and auditory cortex using different pharmacological treatments. We aimed to obtain a correlation between the periphery and the central auditory system. Pharmacological agents used – kainate, pilocarpine and salicylate - were previously shown to alter nerve activity either directly or indirectly. As a starting point, all pharmacological agents were given systemically. Chronic salicylate treatment has previously been shown to lead to behavioural manifestations of tinnitus (Bauer et al. 2001). Using this paradigm, our initial study showed that chronic salicylate treatment downregulated activity-dependent genes in the cochlea but led to an opposite effect in the auditory cortex. Surprisingly, kainate induced similar effects as chronic salicylate treatment while pilocarpine upregulated activity-dependent genes in both cochlea and auditory cortex. These data will be

discussed in the context that BDNF may influence activity patterns in the auditory system.

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Axonal regeneration in adult CNS neurons is promoted by Bcl-XL

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The intrinsic regenerative potential of adult mammalian CNS neurons is limited. Culturing of adult retinal ganglion cells (RGCs) 10 days subsequent to *in vivo* axotomy or squeezing of the optic nerve leads to sparse neurite outgrowth of surviving neurons. Serum deprivation of the culture medium further reduces neurite re-elongation, and adhesion of the retinal explant to the growth permissive substrate is a prerequisite for neurite sprouting. To investigate the antiapoptotic Bcl-2 family member Bcl-XL which remains expressed in adult neurons, for its role in axon regeneration, Bcl-XL content in mature RGCs was increased by adenoviral transgene delivery. Retrograde *in vivo* Bcl-XL overexpression increased both length and numbers of emerging neurites 6.72 ± 0.47 fold and 3.10 ± 0.20 fold over baseline levels, respectively. An even 10.85 ± 0.63 and 3.84 ± 0.30 fold stimulation, respectively, was observed for transduced peripapillary retinal parts. Similarly, gene transfer *in vitro* increased length and number of emanating neurites by a factor of 8.29 ± 0.69 and 5.22 ± 0.41, respectively. Bcl-XL rescue of cultured RGCs from secondary cell death, however, was only slightly pronounced in fields of re-induced axon regeneration, suggesting that Bcl-XL capacity to increase neurite sprouting activity is distinct from its established antiapoptotic function. These results indicate that Bcl-XL might be a major target gene to regulate individual regeneration threshold in adult CNS neurons.

Generation of BAC-transgenic mice using cholinergic- and dopaminergic-specific promoters to express the reverse tetracycline regulated transactivator, rtTA

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The use of the tetracycline-controlled expression system in transgenic mice has become an important tool to study gene function *in vivo*. The ability to reversibly regulate gene expression in a specific cell type should help to identify phenotypic alterations that may provide valuable clues toward understanding a given gene's function.

In an attempt to analyze gene function in cholinergic and dopaminergic neurons, we are in the process of generating transgenic mice that express an improved variant of the reverse tetracycline-controlled transactivator (rtTA) under the control of regulatory sequences from the choline acetyltransferase (ChAT) and the dopamine transporter (DAT) genes. In order to direct rtTA expression that precisely mimics the endogenous expression profiles, we have used large DNA fragments contained in bacterial artificial chromosomes (BACs) as the source of ChAT and DAT-specific promoters and other regulatory elements. The insertion of rtTA-cDNAs into the start codons of the ChAT and DAT genes was accomplished using homologous recombination in bacteria, utilizing a system based on lamda-prophage encoded functions that protect and recombine linear DNA fragments. This method has been demonstrated to work well with short (50 bp) homology arms that can be added to a desired DNA cassette by PCR.

Multiple lines of transgenic founder mice have been generated that are currently being analyzed by breeding to reporter mice expressing both lacZ and green fluorescent protein (GFP) under the control of the tet operator. In addition, we are using *in situ* hybridization and immunohistochemical analyses to determine whether rtTA expression co-localizes with cholinergic and dopaminergic markers. Preliminary results suggest that multiple transgenic lines have been generated that inducibly express rtTA and we are currently determining how faithfully these lines express in either cholinergic or dopaminergic neurons.

978 The survival of motoneuron protein SMN interacts specifically with a high-molecular-weight isoform of fibroblast growth factor - 2 (FGF-2)

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Fibroblast Growth Factor 2 (FGF-2) is an important modulator of cell growth, differentiation and a neurotrophic factor. FGF-2 occurs in isoforms, a low molecular weight 18 kDa and at least two high molecular weight forms (21, 23 kDa), representing alternative translation products from a single mRNA. In addition to its role as an extracellular ligand, FGF-2 localizes to the nuclei of cells. Here we show differential localization of the 18 and the 23 kDa isoforms in the nuclei of rat Schwann cells by means of fluorescence tagging of the individual isoforms with DsRed and GFP, respectively. Whereas the 18 kDa isoform was found in the nucleoli, nucleoplasm and Cajal bodies, the 23 kDa isoform localized in a punctuate pattern and associates with mitotic chromosomes suggesting different functional roles of the isoforms. Moreover, we show here that the 23 kDa FGF-2 isoform is able to specifically interact with the survival of motor neuron protein (SMN). SMN is located in the cytoplasm, in the nucleoplasm and in the gemini of Cajal bodies (gems) and is an assembly and recycling factor of the splicing machinery. Patients with spinal muscular atrophy (SMA) suffer from fatal degeneration of motoneurons due to mutations and deletions of the gene for the survival of motor neuron (SMN) protein.

The effects of polymorphisms in COMT and MAO-A genes on EEG and event related brain potentials (ERPs) 979

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MAO catalyzes the oxidative deamination of a number of biogenic amines, including the key neurotransmitters serotonin (5-HT), norepinephrine (NE), and dopamine (DA). MAO-A enzyme, a form of MAO, plays a critical role in the regulation of catecholamines and indolamine (i.e. especially in norepinephrine and serotonin) neurotransmission. Four variants have been defined in the promoter region of MAO-A gene according to number of the 30-bp repeat sequence (VNTR, Variable Number of Tandem Repeats). Catechol-O-methyltransferase (COMT) is a key enzyme that inactivates catecholamines by catalyzing S-adenosyl-L-methionine dependent to methyl conjugation. The genetic polymorphism of COMT gene results in 3 to 4-fold difference in COMT activity. The differences in COMT activity due to a G to A substitution leading to a valin to methionine substitution produces two variants as high-activity (H) and low-activity (L) variants of the gene coding COMT. In this study, the relationship between the polymorphisms in MAO-A and COMT enzyme genes, and scalp recorded P3a and P3b ERPs were investigated. Additionally, EEG power spectrum analysis was applied for each polymorphism. EEG and ERPs were recorded from forty-eight healthy male volunteers. Auditory oddball paradigm and novelty paradigm were used to obtain P3b and P3a potentials, respectively. Subjects were grouped according to the polymorphism of the gene identified using the PCR method. The amplitudes and latencies of P3a and P3b waves and spectral band powers were measured, and the differences between groups were analyzed by an ANOVA design with the between subject factor, genotype, and two within-subject factors, antero-posterior distribution (frontal, central, parietal) and lateral distribution (left, middle, right). In the present data set, only the most frequently occurring MAO-A gene variants, allele 1 and allele 3, were detected. The only significant difference between these variants was in the lateralization of the P3b latencies. Three genotype groups were defined for COMT polymorphism. It was found that the antero-posterior distribution of P3a latency in HH genotype was significantly different than those in the other genotypes. In spectral analysis, it was found that the α band power of HH genotype was significantly lower over the posterior regions of the scalp, and the δ band power of LH genotype group was relatively higher over the frontal and central sites. Consequently, it was found that polymorphisms in neurotransmitter related enzyme genes, which can change the enzyme activity, might affect the EEG band power and ERPs.

980 Serotonin related gene polymorphisms affect the event related brain potentials (ERPs)

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Event-related potentials (ERPs), which reflect cognitive procesesses of the brain are stimulus or response locked voltage fluctuations on scalp recorded electroencephalography (EEG). These potentials show high inter-individual variability. Psychopharmacological studies have shown that different neurotransmitter systems (i.e. cholinergic, noradrenergic, dopaminergic, and gabaergic systems) are involved in the generation and/or modulation of ERPs. Thus, the variations in genes which are associated with neurotransmitter systems might posses a key role for ERP variety between subjects.

Two gene polymorphisms including serotonin transporter (5-HTT) gene (VNTR and 5-HTTLPR polymorphism) and serotonin 2A receptor (5-HT2A) gene (-1438G/A and T102C polymorphism) were investigated, which affect the serotonergic transmission. Both groups of polymorphisms were associated with major psychiatric disorders such as anxiety, depression, schizophrenia, autism, bipolar affective disorder, and seasonal affective disorder. The aim of our study is to assess whether these polymorphisms may cause any alternation in P300 ERP family. For this purpose, ERPs were recorded from 48 healthy male volunteers. Auditory “oddball” paradigm and novelty paradigm were used to obtain P3b and P3a potentials, respectively. Subjects were grouped according to the polymorphisms identified using the PCR method.

The amplitudes and latencies of P3a and P3b waves were measured by identifying the major positivity in a pre-defined time-window. The effects of the genetic polymorphism on the P3a and P3b amplitudes, latencies and their topographies were analyzed by an ANOVA design with the between subject factor, genotype, and two within-subject factors, anteroposterior distribution (frontal, central, parietal) and lateral distribution (left, middle, right).

In our study, three genotype groups were determined for VNTR polymorphism in 5-HTT gene. Latency of the P3a was found to have more homogeneous topography (i.e. within the same range along the fronto-parietal axis) in heterozygote 12/10 genotype than in homozygote groups (12/12, 10/10) for VNTR polymorphism.

Three variant were determined for 5-HTTLPR polymorphism and the topography of the P3b latency of S/S genotype was found different from S/L and L/L groups. There was no fronto-parietal difference in the P3b latency in the S/S genotype, whereas both S/L and L/L groups had significantly longer P3b latencies in frontal leads compared with those in parietal locations.

Because there is strong linkage disequilibrium between the -1438G/A and T102C polymorphisms, 3 groups that are defined according to these two polymorphisms are almost

the same. As a result, subjects with A/A genotype for -1438G/A polymorphism and T/T genotype for T102C in a parallel manner were found to have different P3a latency topography from other two variants.

Consequently, it is found that possible differences in serotonergic activity because of genetic polymorphisms may affect the ERPs, supporting the suggestion that ERP variability is not only secondary results of behavioral modulations (e.g. arousal, motivation) but also depend on neurobiological variability.

Imaging of neural activity using genetic indicators 981

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Genetically encoded fluorescence indicators based on GFP variants have nowadays been used in multicellular organisms (Kerr et al, 2000; Reiff et al, 2002) to measure changes in local calcium concentrations. Their expression *in vivo* can be directed to cells of choice. This feature qualifies them to analyze single cells or more elaborated sets of neurons in an unstained neuronal environment. Therefore genetic indicators are ideally qualified for investigations of densely packed networks of small neurons as they exist e.g. within the fly visual system (Fischbach and Dittrich, 1989).

In the fruitfly *Drosophila melanogaster* such specific expression can be indirectly achieved by making use of cell-specific Gal4-lines. For this approach appropriate Gal4-lines are required as well as transgenic flies that carry indicator-DNA under control of Gal-4-sensitive upstream activation sequences (UAS) within their genome (Brand and Perrimon, 1993). To finally permit the expression of a genetically encoded indicator within the fly visual system we currently check different Gal-4 expressing lines and generate a reasonable number of new UAS-indicator-flies by P-element mediated transformation. A variety of different types of calcium indicators have been used so far whose working principles are either based on “fluorescent resonance energy transfer” (FRET, e.g. Cameleons, Miyawaki et al, 1997) or the functional constitution of a single chromophore (e.g. Camgaroos, Pericams and G-CaMP, Baird et al, 1999; Nagai et al, 2001; Nakai et al, 2001). In principle these DNA-based indicators can also be targeted to specific sub cellular compartments by fusion to specific localization signals. We work on the construction of such molecules to allow the monitoring of fluorescence signals at locations where calcium-alterations are maximal or difficult to detect with conventional dyes. In a next step we characterize fused and non-fused indicators at the neuromuscular junction of *Drosophila* larvae. The known physiology and easy accessibility for optical and electrical recordings makes this preparation an ideal model system to study indicator characteristics and possible interference with the normal physiology of cells *in vivo* (Reiff et al, 2002). This approach should allow for identifying most suitable indicators and imaging strategies for the future analysis of individual neurons and neuronal networks within the fly visual system.

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982 Modulation of cortical excitability by monoaminergic receptor variants

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Objectives Transcranial magnetic stimulation (TMS) is a feasible tool in neuropsychological research, offering the possibility for the first time to determine cortical excitability *in vivo*. Recently, Wasserman. et al. (2001) could demonstrate a significant correlation between motor cortex excitability and an anxiety-related personality trait, which is decisively influenced by genetic factors. Moreover, sibling data suggest that cortical excitability has a significant genetic input (Wassermann et al., 2002) However, a direct modulation of cortical excitablity by monoaminergic receptor variants has not been shown. Methods Cortical excitability was measured in nine healthy subjects screened for allelic variants in the dopamine D4 receptor gene (DRD4) and the serotonin transporter gene by means of TMS, including MEP threshold and response to paired-pulse TMS. Results A sigificant modulation of intracortical inhibition in volunteers carrying the DRD4*7 repeat allele could be observed. Conclusion This study gives further support to the finding that aspects of cortical excitability may be determined genetically.

983 Identification and characterization of the nc82 antigen, an active zone protein at the presynaptic terminal of dipteran insects

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The monoclonal antibody "nc82" from a *Drosophila* brain hybridoma library (Hofbauer, 1991) is widely used for neuropil staining in confocal microscopy of the *Drosophila* brain (Rein et. al., 2002) due to the high transperency of the preparations. We have used this antibody to stain larval nerve muscle preparations and found that it labels in synaptic boutons as small spots that can be identified as active zones. In insects, only a few synaptic active zone proteins are known so far. We therefore attempted to identify the antigen recognized by MAB nc82. On western blots prepared from *Drosophila* head

homogenates the antibody selectively stains two bands at about 180 and 150 kDA respectively. Purification of the antigen by immuno affinity was unsuccessful due to its insolubility in various non denaturing detergents and an apparent instabilty of the protein. In Coomassie stained 2-D gels of head homogenates, however two isolated protein spots can be identified by blotting and nc82 staining. Mass spectrometry revealed that the two spots presumably represent isoforms translated by alternatively translated mRNAs of the same novel gene of no known function. Blast homology searches detected a homologous gene in the genome of *Anopheles* but no homologies with other species and only a weak but significant similarity with leucine zipper domains of myosine heavy chain proteins from various species was reported. Comparison of *Drosophila* and *Anopheles* genomic sequences made it clear that the annotation of the *Drosophila* gene is incorrect and that the cDNA of the Berkely *Drosophila* Genome project is incomplete. The molecular analysis of the transcription unit by RT-PCR is under way.

Attempts to visualize the nc82 antigen in electron microscopical preparations have met with difficulties due to the fact that fixation of the tissue with gluteraldehyde (GA) at a concentration > 0.06% (GA) destroys the binding of the antibody to its antigen. In our present experiments, structural tissue preservation at 0.06% GA has been insufficient to identify active zones.

An interesting aspect of the nc82 antibody is the fact that it detects a novel phenotype in our cysteine string protein (CSP) mutants (cf. Poster by Schwenkert et al.). In *Csp* null mutants the nc 82 antigen seems to form aggregates which could be responsible for the neurological symptoms of the mutant flies. Since CSPs have been shown to activate the Hsc 70 ATPase (Review Zinsmaier and Bronk, 2001) the nc82 antigen may represent a target molecule of the CSP/Hsc 70 molecular chaperone complex. The function of this novel protein will be studied by genetic knock-down and knock-out experiments.

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Electrophysiological Responses to Cortical Blood-Brain-Barrier Disruption

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Perturbations in the integrity of the Blood-Brain-Barrier (BBB) have been reported in both humans and animal models under numerous pathological conditions. We have recently shown that focal, long-lasting BBB disruption can be imaged in cortical areas of human patients suffering from a wide range of neuropathological conditions. These anatomical findings were associated with neurological symptoms and corresponding focal electroencephalographic abnormalities over the affected cortical areas. However, the detailed physiological changes during acute BBB disruption are not fully understood. Furthermore, it is not known how prolonged disturbances in the extracellular

environment of the cortical network affect its structure and function. Here we report a rat model for long-lasting, focal BBB disruption using dehydrocholic acid (DHC) and 7-Deoxycholic acid (DXC) sodium salts. Recordings from brain slices maintained in-vitro were first performed to determine the concentration ranges in which these agents have no apparent toxicity. For these experiments, electrophysiological responses to afferent stimulation were recorded in the CA1 area of the hippocampus and in layer 2-3 of the somatosensory cortex. DHC up to 5mM and DXC up to 2mM were found to have no significant effect on amplitude, duration and paired-pulse facilitation of the evoked responses. To monitor changes in cortical function exclusively due to BBB disruption we therefore used 1-2mM DHC and 0.5-1mM DXC. Under full anesthesia, 4mm diameter bilateral cranial windows (3mm caudal to Bregma, 5mm lateral to midline) were opened over the somatosensory cortex of adult male rats (ca. ~300g body weight). Disrupting agent was applied for 20-40 minutes on the right somatosensory cortex and saline on the controlled left hemisphere. Using peripheral injection of the non-permeable peptide fluorescein isothiocyanate and the albumin-binding dye Evan's Blue, areas of disrupted BBB could be visualized following the acute experiment to validate the efficiency of our experimental protocol. To monitor the physiological alterations during acute BBB disruption, spontaneous and evoked (to vibrissae stimulation) electrophysiological responses were obtained in-vivo during the disruption procedure. To test whether prolonged compromised BBB leads to changes in cortical function, electrophysiological recordings were made in slices from rats 1-4 weeks following the disrupting procedure. Preliminary results from both experimental procedures are presented.

985 Influence of different promoters on the expression pattern of mutated human α-synuclein in transgenic mice

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Two missense mutations (A53T and A30P) in the gene encoding the presynaptic protein α-synuclein (alphaSyn) are genetically linked to rare familial forms of Parkinson's disease (PD) and its accumulation in Lewy bodies and Lewy neurites. As an initial step to investigate the role of alphaSyn in the pathogenesis of PD, we have generated C57BL/6 transgenic mice overexpressing human alphaSyn. In order to obtain transgenic lines with a significant overexpression in brain regions affected in the course of PD, we used different constitutive promoters to drive the expression of the human mutated alphaSyn (hm alphaSyn):

·mouse tyrosine hydroxylase (msTH)

·human platelet derived growth factor-ß (hmPDGF)

·chicken β-actin (chbact)

·mouse prion protein (msPrP)

In this study we compared the expression level and spatial distribution pattern of the transgene in the various transgenic lines.

Northern and western blot analysis indicate a 1–2fold expression of the hm alphaSyn protein compared to the endogenous alphaSyn. Immunohistochemical analysis with a human-specific antibody against alphaSyn revealed a characteristic transgene expression pattern for each transgenic line. In the lines generated with the msTH promoter the hm alphaSyn was restricted to dopaminergic neurons and some ectopic brain regions, that transiently express TH during development. The hmPDGF promoter induced an overall transgene expression in glial cells, while the distribution pattern in the chbact driven transgenics varied among the lines from an exclusive neuronal, a mixed neuronal-glial to an exclusive glial expression pattern. The msPrP-promoter directed the transgene expression into neurons, that distributed overall in the CNS.

Future studies will analyse how the variations in transgene expression affect the pathogenesis in the animals.

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Mouse pesticide models: Characterisation of neuropathology 986

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Epidemiological studies suggest that pesticide exposure is associated with an increased risk of developing Parkinson`s disease (PD). Recent animal studies have confirmed this destructive effect of pesticides. Thus, chronic infusion of the pesticide rotenone (Rt) with minipumps into Lewis rats emulates many of the pathological phenotypes seen in PD (Betarbet et al. 2000, Nat. Neurosci. 3, 1301-1306), while mice treated with combined paraquat (Pq) and maneb (Mb) exhibit serious lesions in the nigrostriatal system (Thiruchelvam et al. 2000, J. Neurosci. 20, 9207-9214).

The present study was designed (i) to characterise the molecular processes underlying the pesticide-induced neuropathology and (ii) to develop a less labor intensive rotenone-model in mice. Therefore, C57BL76 mice (2-3m) were intraperitoneally (i.p.) injected with either a combination of Pq and Mb (Pq:10 mg/kg// Mb: 30 mg/kg in saline; 2x/w) for 2, 4 or 6w or subcutaneously (s.c.) with Rt (0.5 mg/kg in DMSO/PEG; 2x/d; 5x/w) for 3.5w. Western blot analysis, immunohistochemistry, HPLC analysis and *in situ*-hybridization were used to describe the pathophysiological changes of dopaminergic neurons and glial cells.

None of the applied pesticides caused cell death, as revealed by FluoroJade and silver stain. However, Rt as well as Pq/Mb (2w) injections induced a significant upregulation of dopamine content in the substantia nigra (SN) and striatum (ST) and an increase in the expression level of ND4, a subunit of complex I in SN pars compacta. In contrast to

Rt, Pq/Mb initiated a strong astro- and microgliosis as well as a number of additional gene expression changes, such as the downregulation of the dopamine transporter and ubiquitin C-terminal hydrolase and an upregulation of α-synuclein gene expression in the SN. Interestingly, the latter was found to aggregate in nigral neurons.

These results suggest that the i.p. application of Pq/Mb intoxication induces serious molecular changes in the dopaminergic system, whereas the s.c. application of Rt fails to induce dopaminergic pathology.

987 Analysis of LFP propagation in the hippocampus of epileptic mice

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Temporal lobe epilepsy in humans is associated with a set of anatomical and physiological changes some of which can be reproduced by local injection of kainate into the hippocampus of rats or mice. It was shown that the pathological changes of structure and connectivity observed in the hippocampus of epileptic patients are mimicked more closely in mice than in rats (Bouilleret et al. 99, Neuroscience). It is currently not known how these changes affect the propagation of activity patterns and the local excitability of the hippocampal network. We compared the induction and propagation of local field potential responses (LFP) in acute slices of the hippocampus from normal, saline-injected and kainate-injected mice.

Kainate was injected into the dorsal hippocampus of one hemisphere of C57/Bl 6 mice. Persistent and stable epilepsy developed across a period of 4-6 weeks after injection. After this period, we prepared transverse slices (400 µm) from ± 1 mm around the injection site. LFP responses were elicited by electrical stimulation and recorded with microelectrode arrays (60 electrodes).

Stimulation of the Schaffer collaterals induced no or much smaller postsynaptic LFP responses in CA1 in slices from kainate-injected hippocampi (KHS) than in slices from normal or saline injected hippocampi (NHS & SHS resp.). This is in agreement with reports that pyramidal cells, and inhibitory interneurons (IN) are mostly lost in this area in KHS. Mossy fiber (MF) stimulation in KHS elicited small but significant responses in CA3. Residual postsynaptic responses are of the same general shape as in NHS, suggesting that rewiring does not take place. Stimulation of the DG resulted in multiple postsynaptic peaks in the DG and the hilus indicating recurrent excitation. This is consistent with findings that MF sprout to form collaterals projecting back onto the granule cell (gc) dendrites and that IN and polymorphic cells in the hilus are lost in KHS.

NHS could usually not be provoked to generate spontaneous epileptiform spikes by disinhibition. Electrical stimulation of disinhibited NHS and SHS, however, led to sin-

gle, enlarged responses propagating through the slice (bicuculline, 25 µm/l). In contrast, such responses could not be induced in KHS at bicuculline concentrations up to 40 µm/.

The generation of epileptic seizure in kainate-injected mice suggests that the observed anatomical and functional changes lead to increased excitability and/or facilitated propagation of excitation in the hippocampus. The results described above indicate that propagation is not enhanced along the hippocampal lamellae *in vitro*. Recurrent excitation of the gc could, however, implement a locally enhanced excitability. EEG recordings indicated that the epileptic activity does not generalize across the cortex in most cases in these mice (Riban et al. 2002, Neuroscience), which would be compatible with the *in vitro* results.

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BAG1 over-expression in brain protects against stroke 988

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The co-chaperone BAG1 binds and regulates 70 kDa heat shock proteins (Hsp70/Hsc70) and exhibits cytoprotective activity in cell culture models. Recently, we observed that BAG1 expression is induced during neuronal differentiation in the developing brain. However, the *in vivo* effects of BAG1 during development and after maturation of the central nervous system have never been examined. We generated transgenic mice over-expressing BAG1 in neurons. While brain development was essentially normal, cultured cortical neurons from transgenic animals exhibited resistance to glutamate-induced, apoptotic neuronal death. Moreover, in an *in vivo* stroke model involving transient middle cerebral artery occlusion, BAG1 transgenic mice demonstrated decreased mortality and substantially reduced infarct volumes compared to wild-type littermates. Interestingly, brain tissue from BAG1 transgenic mice contained higher levels of neuroprotective Hsp70/Hsc70 protein but not mRNA, suggesting a potential mechanism whereby BAG1 exerts its anti-apoptotic effects. In summary, BAG1 displays potent neuroprotective activity *in vivo* against stroke, and therefore represents an interesting target for developing new therapeutic strategies for reducing brain injury during cerebral ischemia.

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989 Parkinson in fruitfly, gene expression pattern changing during time?

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Parkinson´s disease (PD), a common neurodegenerative dosierter, is characterized by loss of dopaminergic cells in the substantia nigra pars compacta. The loss of these cells is along with formation of filamentous intraneuronal inclusions called Lewy bodies in which the protein α-synuclein accumulates. Several types of α-Synuclein identified: The wild-type form and family forms as A53T, or A30T .The clinical manifestations of PD are bradykinesia, rest tremor and rigidity.

There are several experimental models of PD expressing α-Synuclein in neuronal and especially dopaminergic cells, as *Drosophila melanogaster* or mouse. We use the GAL4-expression system, because it is well established and shown that there are α-synuclein-immunostainable inclusions and distinct changes in behaviour of transgenic fruitflies. The short lifespan of *Drosophila melanogaster* makes it possible to reveal changes of the expression-patterns during lifetime in short intervals.

After crossing the GAL4-driver line with a α-synuclein effector line we sample adult individuals at different stages of age, dry them in acetone to make the mRNA-population save, prepare the part of interest of nervous system and isolate the mRNA. We capture the mRNA with polysterine-avidine beads coupled to poly-dT-nucleotides. After amplification and labelling with fluorescent molecules, we hybridize the cDNA/cRNA to *Drosophila*-cDNA-chips. Therefore, we use several cDNA chips as 7k-Canadian- or 14k-Heidelberg Chips. We also use self-made signal-transduction-chips. The read out chips show the expression patterns and will be compared with that of wild type-flies. We will focus on genes which are normally not expressed in wild type flies but coincidentally with α-synuclein, such as chaperones and others.

990 The expression of $GABA_A$- and AMPA-receptor mRNA in the primary motor cortex of patients with amyotrophic lateral sclerosis

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The pathomechanism of amyotrophic lateral sclerosis (ALS) still remains unclear to date. This progressive neurodegenerative disorder affects upper as well as lower motor neurons and is characterized by a combination of muscular wasting and spasticity. There is some evidence that excitotoxic cell death is involved in the degeneration of the motor nervous system and that ligand-gated receptor channels play a role in the pathogenesis of the disease. An imbalance of inhibitory and excitatory influences might lead to chro-

nic neurotoxicity by a chronic imbalance of intracellular calcium concentrations. Several electrophysiological and anatomical studies support the pathophysiological concept of an impaired inhibitory, namely GABAergic, control of the motoneurons in the cerebral cortex of ALS patients. There is also some evidence for alterations in the excitatory synaptic transmission via AMPA type glutamate receptors in the motor system in ALS. The aim of our study was to investigate the expression of the mRNAs of 14 $GABA_A$-receptor subunits and of the four AMPA type glutamate receptor subtypes in the motor cortex of ALS patients compared to control persons. We therefore performed *in situ* hybridization histochemistry (ISH) and immunohistochemistry on human post mortem motor cortex sections of ALS patients (n=5) and age matched controls with no history of neurological disease (n=5). The mRNA expression was quantified macroscopically by densitometric analysis of digitized film autoradiographs, the receptor expression at the cellular level was studied by liquid emulsion autoradiography.

The most intriguing finding was a significantly reduced mRNA expression of the α1-subunit of the $GABA_A$-receptor in ALS patients while the level of the $GABA_A$-receptor β1-subunit mRNA was elevated in the patients group. All other subunits investigated showed no differences at the transcriptional level. This may indicate specific alterations of the $GABA_A$-receptor subunit composition and result in distinct physiological and pharmacological properties of these receptors in ALS patients. Whether this is a principal mechanism in the pathophysiology of ALS or a secondary adaptive mechanism during the disease process has to be elucidated in further studies.

Interneurons respond actively upon cortical changes in the Val12-Ha-ras transgenic mice

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The effect of a changed cortical architecture upon interneurons has been investigated in the primary sensory cortex of the Val12-Ha-ras transgenic mice using GABA, parvalbumin, calbindin, calretinin and VGAT (vesicular GABA transporter) immunostainings. In this heterozygous transgenic line p21H-rasVal12 is postnatally expressed in pyramidal cells around day 15, when most of the neurones are postmitotic and synaptic contacts have been established, which results in permanent expression of the ras protein in pyramidal neurons. The volume of cerebral cortex has been reported to be increased by approximately 20%, which has been suggested to be the consequence of an increased volume of cortical pyramidal cells since their number remains unchanged. Local circuit neurons neither increased their perikaryal size nor their relative density, and they preserved their main morphological and immunhistochemical features. The dendritic arbour, however, was observed to follow the cortical expansion in many cases. Changes in the synaptic pool established by interneurons was investigated by using a laser scanning microscope on VGAT immunostained sections. The results of the present study show,

that postmitotic interneurons react actively upon changes in cortical architecture in Val12-Ha-ras transgenic mice by altering the features of their processes thus preserving their fundamental role in cortical functioning.

992 Aggregation of phospho-Tau-analoga induced by heparin, aluminum-ions and iron-ions

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Alzheimer´s disease is neuropathologically characterized by neurofibrillary tangles which are made up by bundles of paired helical filaments whose main constituent is the abnormally phosphorylated protein Tau. A profoundly increased phosphorylation state of Tau has been implicated to be on of the important events in the pathogenic cascade leading to Tau aggregation. To determine the potential role of Tau hyperphosphorylation mutated Tau proteins were generated. Serine and threonine residues were substituted for glutamate and aspartate and clustered in different constucts to introduce negative charges and to simulate hyperphosphorylation of Tau protein on specific sites. We tested the ability of mutated Tau and wildtype Tau to undergo filament formation primed by different substances like heparin, aluminum-ions and iron-ions. Constructs, mimicking phosphorylation in the N-terminal half and the proline-rich region show a decreased ability to undergo filament formation by presence of heparin, aluminum-ions and iron(III)-ions. Constructs with mutation in the microtubule-binding region of Tau do not differ from wildtype-Tau. The present paradigm provides an experimental model for analysing the functional consequences of Tau hyperphosphorylation and its effects on aggregation critically involved in the pathomechanism of AD.

993 Expression of cell cycle regulators in the developing mouse brain

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The development of the central nervous system requires a strict control of cell cycle entry and withdrawl. In mammalian brain, neurons withdraw from the cell cycle immediately after their differentiation from proliferative neuroepithelial cells. During the ontogenesis of the brain the expression, assembly and activation of specific cyclins and cyclin-dependent kinases (cdks) are required in a tightly regulated, sequential fashion. Cell cycle is arrested mainly by molecules which inhibit cdks. Cdk inhibitors include members of the Ink4 familiy and the Cip/Kip familiy. Little is known about the expression pattern of cyclins, cdks and cdks inhibitors during the development of the mouse brain.

In the present study we investigate by immunohistochemical and biochemical methods, which cell cycle regulators are involved in the control of neurogenesis. Stage-specific expression of cyclins and cdks was examined in brain tissue of mice from embryonic

day 14 to postnatal day 11. Immunohistochemical observations indicate that expression of cyclins (D, E, A, B) is augmented at days E14 – E17. The expression pattern of the cell cycle regulators is time-specific. At embryonic days D-type cyclins are found preferentially in the ventricular zone of the 3th, 4th and the lateral ventricles. The expression is downregulated in the ventricular zone after birth but increases in neurons of non-proliferating regions of the brain. Cyclins A and B are expressed more ubiquitously throughout the brain until day P11. However, expression is decreased at E 17 and even more at P 11 within the neuroepithelium while persists in other regions. Furthermore, the immunohistochemical characterisation showed that proteinkinases cdc 2 and cdk 4 are constitutively expressed without notable changes until P11. Cdc 2 is expressed in all brain regions. Within the olfactory bulb, cyclins and cdks are upregulated constantly during pre- and postnatal stages. To verify the immunohistochemical observations, cyclin expression was quantified by means of ELISA. The data suggest that levels of some cell cycle related proteins varies during developmental progression whereas others are expressed constantly.

This study indicates, that cell cycle regulatory proteins are differentially expressed in the mouse brain in a developmental stage-dependent manner with elevated levels in the neuroepithelium.

Perineuronal nets potentially protect against oxidative stress 994

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Perineuronal nets are a special form of extracellular matrix and consist of large aggregating chondroitin sulphate proteoglycans connected to hyaluronan as main components. Perineuronal nets surround different subpopulations of neurons. Due to their glycosaminoglycan components, the perineuronal nets form highly negative charged structures in the immediate microenvironment of neurons and might be involved in local ion homeostasis. Perineuronal nets might also potentially be able to scavenge and bind redox-active iron, and to reduce the local oxidative potential in the neuronal microenviroment, thus providing some neuroprotection to net associated neurons. Here, we show that neurons enwrapped by a perineuronal net in the human cerebral cortex are less frequently affected by lipofuscin accumulation than neurons devoid of a net both in normal aged brain and Alzheimer´s disease. As lipofuscin is an intralysosomal pigment mainly composed of cross-linked proteins and lipids generated through iron-catalyzed oxidative processes, this study proposes a possible neuroprotective effect of perineuronal nets against oxidative stress potentially involved in the pathomechanism of Alzheimer´s disease and related disorders.

995 Cell cycle and cell death in Alzheimer´s disease: The role of cyclin B and cdk 1

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Recent studies show that mechanisms of cell cycle activation are critically involved in cell death in Alzheimer´s disease. Cell cycle proteins, such as cyclins and cyclin dependent kinases (cdk), have been identified in degenerating neurons in Alzheimer´s disease. In differentiated cells, such as post-mitotic neurons, the re-entrance into the cell division cycle cause cell cycle arrest, apoptosis and cell death. In proliferating cells, cyclin B and the associated kinase cdk 1 control the transition from G2-phase into mitosis. Whereas neurons in Alzheimer´s disease express cyclin B and cdk 1, no mitotic states have ever been observed. This brings up the question if the unsheduled cyclin B expression in differentiated cells does affect cell death.

To study the effects of cyclin B expression on cellular viability, we expressed cyclin B in the neuroblastoma cell line SH-SY5Y using a tetracycline-controlled gene expression system (Tet-On). SH-SY5Y-Tet-On cell clones were generated and characterized, chosing a clone with high induction and low background expression for further experiments. SH-SY5Y-Tet-On cells were differentiated into neuron-like cells using BDNF, Retinoic Acid, Herbimycin A or phorbol ester. Differentiated cells were transfected with pBI plasmids and the expression of cyclin B was induced adding doxycycline. For investigation of the molecular and cell biological mechanisms of cyclin B expression in neuron-like differentiated cells the content of cyclin B was measured by FACS and the active cdk 1 was analysed by kinase assay.

996 The cdk5-activators $p25^{nck5a}$ and $p35^{nck5a}$ contribute to cell death of SH-SY5Y neuroblastoma cells

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Cycline-dependent kinase 5 (cdk5) is termed according to its structural resemblance to other cdks, but has no known function in the cell cycle. It displays effects in postmitotic cells and opposed to its name frequently interacts with non-cycline activators. An activator for cdk5 is the neuronal cycline-dependent kinase 5 activator (nck5a) p35 as well as the truncated form p25, generated by calpain-cleavage of $p35^{nck5a}$. As in neurodegenerative conditions such as Alzheimer's disease $p25^{nck5a}$/$p35^{nck5a}$ are potentially involved in apoptosis, in the present study we analysed their effects on the viability of neuroblastoma cells.

We cloned the human genes for $p25^{nck5a}$ and $p35^{nck5a}$, respectively, in plasmid vectors either separately or as fusion genes with the enhanced green fluorescent protein (EGFP) sequence. The human neuroblastoma cell line SH-SY5Y was transfected with the $p35^{nck5a}$-EGFP, the $p25^{nck5a}$ and the $p35^{nck5a}$ vector, respectively. Additionally, African

green monkey kidney COS-7 and Chinese hamster ovary CHO-K1 cells were transfected with $p25^{nck5a}$/$p35^{nck5a}$.

The elevated expression of $p25^{nck5a}$, $p35^{nck5a}$ or $p35^{nck5a}$-EGFP caused an increasing number of SH-SY5Y cells to die, while in COS-7 and CHO-K1 cells elevated levels of the activators showed no effects on cellular viability.

These results indicate that high levels of $p25^{nck5a}$/$p35^{nck5a}$ cause loss of adhesion and apoptosis particularly in neurons.

Cajal-Retzius cells in normal aging and Alzheimer's disease: The entorhinal cortex 997

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Neurodegeneration in Alzheimer´s disease is associated with aberrancies in synaptic plasticity and neuronal repair. Several trophic molecules that play key roles during normal embryonic and fetal brain development are re-activated during the course of the disease. One of them is the large glycoprotein Reelin that is produced and secreted by Cajal-Retzius cells located in the marginal zone, the later cortical layer I. Reelin assures correct layering of the neocortex. Several neuropsychiatric disorders have recently been found to be associated with abberancies in the Reelin/ Cajal-Retzius cell system. Reelin has been postulated to act on the downstream target protein Disabled-1. Disturbances of this pathway interfere with cytosceletal hyperphosphorylation, presenilin and amyloid precursor protein signalling as well as Cdk-5 mediated mechanisms known to play also a role in the pathogenetic cascade of Alzheimer's disease. Our own results show that Cajal-Retzius cells are preserved in Alzheimer's disease. They maintain their morphological heterogeneity in layer I of entorhinal cortex in the normal senescent brain and in Alzheimer's disease. Few Reelin-immunoreactive Cajal-Retzius cells display immunoreactivity for paired helical filaments - typical for pyramidal cells in Alzheimer's disease. Co-localization of β-Amyloid and Reelin has not been revealed using immunohistochemistry. The facts that Cajal-Retzius cells are preserved in Alzheimer's disease and that the majority of them displays no signs of degeneration indicates normal function.

998 Glioma cell migration along adult neurites – a new *in-vitro* model

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Glial cells of the brain can turn in to malignant tumor cells, representating 25% of the cns tumors. The unchanged worse prognosis in the therapy of these tumors such as glioblastoma gives rise to the investigation of the behaviour of these tumors on migration and proliferation. In this presentation we'll show a co-culture in-vitro model of regenerated axons from the retinal ganglion cells of rats and the C6 glioma cell line, further the effect of interfering anti-α6integrine antibodies on the glioma-axonal interaction.

method: Adult rats (6-8 weeks old) were operated (open crush and lens injury) at the optic nerve 5 days before retina explantation to stimulate neurite regeneration. To perform the open crush the orbita is opened (the m.levator palpebrae, mm. recti sup. et lat., m. retractor bulbi were cut near insertion) and the intraorbital fat is removed to approach the optic nerve. The dura mater is spliced in a way sparing the retinal artery. The open crush is performed with fine forceps. Lens injury was made with a small size needle passing through sclera and chondroid The retina was explanted as described earlier on petriperm dishes coated with poly-D-lysine and laminin with S4 medium for neuronal cells created in our laboratory based on DMEM with enriched glucose and oxygen. After 2-3 days neurite growth is sufficient for co-culture with C6 glioma cells. After cell counting C6 glioma were seed on the petri dishes using glass capillaries with 5mm height. A dish is put in an on-microscope-incubator chamber holding the atmosphere at 20% oxygen and 5% carbondioxide.

group A dishes were added 1ml 1:5 supernatant of anti-α6-integrine antibody proven to label the α6-integrine by immunohistochemistry. The group B dishes were added 1ml 1:5 (Iscoves MEM+5%FCS) as control.

Evaluation: Migration behaviour of C6 Glioma cells on neurites is registered by velocity and direction of movement relative to the neurites. Comparison between group A and B allow us to describe the role of α6-integrine on migration of the C6Glioma cells.

Results: The blockade of a6Integrine in-vitro showed only short results. Group B behaviour is similar to A. We suggest that the C6 Glioma cells are capable to evade the effect of a6integrine blockage through expression of other surface molecules. Further integrin subunits like α5 and α3 integrins will be investigated in a similar way.

Endogenous antiepileptic processes are activated by epileptiform activity in a model nervous system

999

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Mechanisms underlying the termination of a seizure are largely obscure. It has often been suggested that epileptiform activity itself leads to its suppression. This suggestion was presently studied using a model nervous system.

The buccal ganglia of *Helix pomatia* were used as model system. The ganglia were kept in an experimental chamber (volume ca. 1 ml) which was continuously perfused with a snail "Ringer" solution (1 ml/min to 3 ml/min). Both neurons B3 in the paired ganglia were impaled with microelectrodes which contained 150 mM KCl. To shift membrane potential by current injection, a second microelectrode was inserted into the soma. Epileptiform activity (paroxysmal depolarization shifts, PDS) was induced with pentylenetetrazol (40 mM).

During a constant superfusion of the ganglia for 24 h with a solution containing the epileptogenic drug, epileptiform activity appeared reliably in both neurons B3. Activity was maximal during the first hour of treatment. During the following 3 h to 15 h, epileptiform activity decreased continuously often down to zero level. After this suppression some epileptiform potentials reappeared which demonstrated that the suppression was no "run down".

To study whether the epileptiform activity of a neuron leads to the suppression of its epileptiform activity, one of both neurons B3 was hyperpolarized to above -100 mV to prevent PDS. The second neuron B3 served as control. When in the control neuron the suppression of PDS became obvious, the test neuron was released from hyperpolarization. The epileptiform activities of the test neuron following its release from hyperpolarization were comparable to those of control neuron following the begin of the treatment with pentylenetetrazol. Thus, the endogenous suppression was missing without epileptiform activity.

With respect to mechanisms underlying the endogenous suppression, some pilot experiments were done concerning a role of protein kinases. To activate PKA, forskolin (50 µm) was added to the bath solution. With application of forskolin, the suppression was blocked. This block was also detectable when forskolin was applied during control conditions prior to the application of the epileptogenic drug.

Furtheron it was tested, whether the suppression depended upon the ionic composition of the bath fluid. It was found that the suppression needed Mg^{2+}. Zero-Mg^{2+} solution resulted in a constant high PDS-activity when applied at the begin of a treatment. When applied during maximal suppression, the suppression was blocked. This means that non-epileptic activity for hours during the suppression was replaced by full blown PDS-activity with application of zero-Mg^{2+} solution. Mg^{2+} could be replaced by high amounts of Ca^{2+} but not by Sr^{2+}.

Results suggest that epileptiform activity suppresses itself in the single neurons via processes which depend upon Mg^{2+} and which are counteracted by PKA. Zero-Mg^{2+} epileptiform activity migth result from a block of endogenous suppression.

1000 Neuronal pacemaker potentials develop into epileptiform activity in model nervous systems

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The physiologic basis of epileptic activity in the nervous system is still not fully known. Especially the role of extra-synaptic processes to the generation of epileptic potentials (i.e. paroxysmal depolarization shifts, PDS) are a matter of controversy. Presently it will be demonstrated that PDS in a model nervous system develop from pacemaker potentials without contribution of synaptic processes.

Identified neurons in the buccal ganglia of *Helix pomatia* and in the abdominal ganglia of *Aplysia californica* were used. Several neurons were recorded simultaneously using intracellular microelectrodes. Epileptiform activity was induced by application of pentylenetetrazol (1 mM to 100 mM) or etomidate (0.1 mM to 1.0 mM). Membrane potentials was shifted via second intracellular microelectrodes.

Pacemaker potentials in the abdominal ganglia of *Aplysia californica* are well known. Pacemaker potentials in the buccal ganglia of *Helix pomatia* appeared regularly in both neurons B3 when membrane potential was shifted to between -50 mV and -40 mV. Frequency of occurrence of pacemaker potentials was about 1/s. The potentials consisted of a primary depolarization which turned into a secondary one which was superimposed by one or several action potentials and which was terminated by a repolarization. Since the pacemaker potentials only appeared within a small window of membrane potentials and since frequency of pacemaker potentials in a neuron increased with depolarization, they were likely generated non-synaptically.

Pacemaker potentials developed into PDS with increasing concentration of an epileptogenic drug. The main process underlying this transformation was a delay of repolarization. Additionally, the potentials increased slightly in amplitude. Only neurons of the used ganglia which could generate pacemaker potentials could also generate PDS. The activation characteristics of the potentials were tested using slow (12 min) triangular currents. With increasing concentration of an epileptogenic drug the window of activation widened to more negative membrane potentials. With epileptogenic concentration (pentylenetetrazol: 40 mM, etomidate: 0.6 mM), PDS even appeared at membrane potentials of -100 mV.

Using the buccal ganglia of *Helix pomatia* it was additionally tested whether chemical synaptic activities contributed to the generation of PDS. All EPSP and IPSP were suppressed dose dependently by the used epileptogenic drugs. With epileptogenic concentrations, IPSP were completely blocked wherease EPSP were reduced to 10% of initial values. Some neurons in the abdominal ganglia of *Aplysia californica* and in the

buccal ganglia of *Helix pomatia* are known to be strongly driven from chemical synaptic inputs. These neurons did show neither pacemaker potentials nor PDS.

Although the mechanisms underlying the development of pacemaker potentials into PDS remains obscure, the processes which establish and maintain pacemaker potentials are decisive for establishment and maintenance of epileptiform activity.

Proteolytic enzymes trigger epileptogenic properties in a model nervous system 1001

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Proteolytic enzymes are often used as a tool to facilitate the mechanical isolation of single neurons or to soften connective tissue for microelectrode impalement. The enzymes are known to leave neurons largely unaffected if they stay in the extracellular space. It is presently shown that epileptiform activities of neurons increase during several hours following a treatment with proteolytic enzymes.

The buccal ganglia of *Helix pomatia* were used as a model nervous system. Both neurons B3 in the paired buccal ganglia were impaled with microelectrodes which contained a 150 mM KCl solution. Epileptiform activity (paroxysmal depolarization shifts, PDS) were induced with pentylenetetrazol (5 mM to 40 mM). Proteolytic enzymes (Pronase, Merck) were applied for 5 min to 3 h in concentrations of 0.1% to 1.0%.

In control experiments, pentylenetetrazol was applied for 24 h. With application of 40 mM pentylenetetrazol, PDS appeared reliably in neurons B3 whereas 10 mM of pentylenetetrazol only induced "inter-iktual" short activity bursts. During the continuous application of pentylenetetrazol, the epileptiform activity showed typical alterations: Activity was maximal during 1 h following the begin of the treatment. Activity then decreased often to zero level. After this endogenous suppression, the epileptiform activity reappeared spontaneously but stayed on a lower level than in the beginning of a treatment with pentylenetetrazol.

In test experiments, proteolytic enzymes were added to the solution in differential latencies to the begin of an application of pentylenetetrazol. During application of the enzymes, the epileptiform activity was blocked or it was accelerated. These direct effects were not considered since the exact composition of the mixture of enzymes is unknown.

With washing out the enzymes, epileptiform activities increased in intensity during several hours. When 1% of proteolytic enzymes were applied for 5 min, epileptiform activities increased more than twofold during an average of 7,4 h (min: 0.3 h, max: 20 h, n=16). In most cases the epileptiform activity was maximal in the end of a 14 h experiment. To study whether the treatment with proteolytic enzymes can activate endogenous epileptogenic properties, the experiments were repeated using sub-threshold concentrations of pentylenetetrazol (5 mM to 10 mM). The appearance of typical full blown PDS depended upon concentration of proteolytic enzymes, duration of their application and the concentration of pentylenetetrazol. With 0.3% of enzymes applied for 30 min and with 8 mM of pentylenetetrazol, PDS appeared after 19 h (average value, n=4). Epilepti-

form activities appeared after 7 h (average value, n=11) when the enzymes (0.3%; pentylenetetrazol 8 mM) were applied for 60 min and PDS appeared after 5 h (average value n=15) with 10 mM of pentylenetetrazol (0.3% enzymes for 60 min).

As a whole, experiments suggest that proteolytic enzymes can induce epileptogenic properties which develop within several hours without obvious further presence of the enzymes.

1002 Detection of abnormal prion protein in sporadic Creutzfeldt-Jakob disease (sCJD)

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A definite diagnosis of Creutzfeldt-Jakob disease (CJD) can be made only by neuropathology or immunochemical demonstration of the pathologic isoform of the prion protein (PrP^{Sc}) in human brain tissue. There is considerable interest in developing methods to detect early infection before clinical signs appear. The aim of our study was to explore the possible use of capillary electrophoresis (CE) for detection of PrP^{Sc} and to investigate whether PrP^{Sc} can be identified with CE in cerebrospinal fluid (CSF) samples from patients with sporadic Creutzfeldt-Jakob disease (sCJD).

CSF samples from 205 patients with definite (neuropathologically confirmed) and probable sCJD and controls with various other dementias were analyzed. 1 ml of human CSF was used for extraction. Each CSF sample was treated after DNAse incubation with proteinase K for 1 h at 37°C followed by PrP^{Sc} extraction protocol [Schmerr et al, 1999] (covered by US Patent 6,150,172). The eluted samples were dried in a vacuum centrifuge at room temperature and then re-suspended in CE-running buffer for testing. Analyses were performed using the P/ACE™ MDQ from Beckman Coulter equipped with a detector for laser-induced fluorescence (LIF). Bare fused-silica capillaries were used. The capillary preparation included pre-treatment with 0,1 N NaOH, followed by a rinse with running buffer. The sample was injected hydrodynamically followed by an injection of the running buffer. To determine the amount of antibody that bounds 50% of the fluorescence labelled peptide, varying amounts of purified antibody were mixed with labelled peptide at 25°C and were measured directly of after incubation for 12-16 h at 4°C.

In total 205 CSF samples were analyzed for abnormal prion protein by fluorescent immunoassay. In 97 cases the results were not readable due to technical problems. The remaining 108 CSF samples included 48 patient with sCJD and 60 controls with various kinds of dementia. In sCJD patients, 25 out of 47 were tested positive (sensitivity: 53%). All controls with different types of dementia were tested negative (specificity: 100%).

First results using CE in CSF indicate different electropherogram pattern in patients with sCJD and controls. These results in sCJD patients indicate that the extraction and testing method could serve as a means of differentiating CJD from other similar diseases. The

method needs further development and optimization but holds promise for diagnosis of CJD in humans.

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IL-8 and IL-8 receptors are expressed in cultured glial Müller 1003 cells from guinea pig and human retinae

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Interleukin-8 (IL-8) is a proinflammatory chemokine. Activation of IL-8 receptors causes migration, exocytosis and a respiratory burst in neutrophils. Several diseases of the eye (e.g. uveitis, melanoma) are associated with an increase of the intravitreal concentration of IL-8. Several cell types, such as retinal pigment epithelium cells (RPE), fibroblasts or ciliary epithelial cells, are potential sources of the secreted IL-8. The aim of our study was to evaluate whether retinal glial cells are able to produce and secrete IL-8 and/or to express IL-8 receptors (IL-8R). We used primary cultures of isolated Müller cells from guinea pig and human retinae. The guinea pig Müller cells were stimulated with the supernatants of human melanoma, RPE and HeLa cells. The Müller cell cultures were prepared for immunocytochemistry, western blotting and RT-PCR, and the supernatants were used for ELISA analysis. Ca^{2+} imaging was performed to test the activation of IL-8 receptors. IL-8 mRNA could be detected in control and stimulated Müller cell cultures. IL-8 immunoreactivity was accompanied by GFAP immunoreactivity in all cells from stimulated and non-stimulated cultures. ELISA analysis of the supernatants revealed that IL-8 was secreted from Müller cells into the culture medium. Nearly all Müller cells in the control and stimulated cultures were more or less intensively immunolabeled with the IL-8RA and IL-8RB (CXCR1 and CXCR2) specific antibodies. Western blot analysis confirmed the expression of IL-8RA and IL-8RB in Müller cells. Application of recombinant IL-8 protein to the Müller cells caused an increase in intracellular Ca^{2+} levels in subpoulations of the cells. Ca^{2+} responses could be detected in 5% of the control cells, in 1% of the melanoma-stimulated Müller cells, and in 23% of the RPE stimulated Müller cells. We never observed Ca^{2+} responses in HeLa-stimulated Müller cells. The expression of IL-8, IL-8RA and RB was also demonstrated in primary Müller cell cultures from human donors, by using immunocytochemistry. We show, for the first time, that retinal Müller glial cells can produce and secrete IL-8 and that these cells co-express IL-8 receptor CXCR1 and CXCR2 under culture conditions. It is concluded that Müller cells may participate in the inflammatory response of pathologically altered or injured eyes.

1004 Relationship between endogenous levels of cytokine mRNA in the striatum and anxiety-like behavior in the rat

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Our previous experiments have shown that adult male Wistar rats can differ systematically in plus-maze behavior, which was related to the neurotransmitter serotonin in the ventral striatum. The plus-maze serves as a model of anxiety-like behavior, and there is evidence that interleukin (IL)-2 in the brain may be related to anxiety-like behavior, and that IL-2 interacts with the striatal serotonergic system. We asked whether plus-maze behavior may also be related to endogenous levels of cytokines in the striatum. Based on the percentage of open arm time in the elevated plus-maze, male Wistar rats were divided into sub-groups with either low, or high, anxiety-like behavior. Then, IL-1β, IL-2, IL-6, and tumor necrosis factor (TNF)-α cDNA levels were measured post mortem in striatal tissues using semi-quantitative, competitive, reverse transcription polymerase chain reaction (RT-PCR). Rats with high anxiety-like behavior in the plus-maze showed significantly higher levels of IL-2 mRNA compared to those with low anxiety-like behavior, but did not differ significantly in expression of IL-1β, IL-6, and TNF-α mRNA. These results provide new evidence indicating that specific cytokine patterns in the striatum may be associated with plus-maze behavior in adult male Wistar rats.

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1005 Interferon-β affects neocortical neuronal activity and excitability

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Interferon-β (IFN-β), a cytokine with antiviral, antiproliferative and immunomodulatory potential, is one of the most effective drugs in the treatment of multiple sclerosis. Effects of IFN-β on neuronal functions are unravelled so far. We investigated the properties of neocortical somatosensory layer 2/3 and 5 neurons by the use of intracellular sharp microelectrode recordings upon application of IFN-β in the concentration range of 1 - 1,000 U/ml. Starting at the concentration of 10 U/ml, which is equivalent to 1 - 10% of the commonly used immunomodulatory dosis, IFN-β increased the excitability in cortical neurons in two kinetically distinct, independent manners, firstly by reversibly influencing the resting membrane properties and secondly by irreversibly enhancing the rate of action potential firing. The resting membrane properties, i. e. membrane resistance R_M and time constant τ of the neurons, were raised 2.5-fold and 1.7-fold, respectively, in a dose-dependent manner (ED_{50}=14.7 for R_M and ED_{50}=9.9 for τ, respectively). This led

to an augmented subthreshold signal propagation. Whilst the effect on R_M and τ developed completely within 30 minutes of superfusion, the dose-dependent enhancement of the firing frequency in a subset of 72% of the neurons established for a longer period, even if the effect on R_M and τ had been fully reversed following washout of IFN-β. Regarding both effects there was no layer specificity. In conclusion, IFN-β plays a pivotal role in the regulation of the neuronal excitability in neocortical pyramidal neurons, which may contribute to the drug's effects and/or side effects in the central nervous system *in vivo*.

A link between the nervous system and the immune system in insects? 1006

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In vertebrates, it is well established that there are many intricate interactions between the immune system and the nervous system and vice versa (Ader et al. 1991; Maier et al. 1994). Regarding insects, till now it has never been asked whether there is a link between these both systems. Here we will present behavioral evidence indicating a link between the immune system and the nervous system.

Using the well-established classical conditioning procedure of the proboscis extension paradigm in honeybees we investigated whether there is any effect of an immune response on memory formation. We challenged the bee's immune system by injecting, into the hemolymph, a dose of lipopolysaccharides, a highly immunogenic but non-pathogenic elicitor. Additionally two control groups were used (a) bees that were injected with Ringer solution alone and (b) sham-manipulated bees with no injection. All the bees were exposed to one conditioning trial and either tested at 1min or 12 min after the conditioning trial.

We found no differences in the responses between the three groups when tested at 1 minute. Activating the immune response with LPS has no effect on general sensory processing and motor activity. Additionally it does not reduce the non-associative sensitization effect of the sugar water stimulation. However, at 12 minutes the LPS treated bees show a significant lower response level then the two control groups ($\chi^2 = 8.81$.d.f. $=2$, $p = 0.012$) .

As LPS has no pathogenic effects, our results suggest that the activation of the immune response itself somehow interferes with memory consolidation. Future work will attempt to elucidate the biochemical links between the immune and nervous system in insects.

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1007 Affective properties of 1,25-$(OH)_2$-vitamin D_3 and other steroid hormones in rats with or without exposure to endocrine disruptors

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The purpose of the present experiments was a) to examine if the active form of vitamin D_3, 1,25-$(OH)_2$-vitamin D_3 (1,25-VD), has affective stimulus properties like other steroid hormones, e.g. 17-β-estradiol or testosterone, and b) to investigate if affective properties are influenced by developmental exposure to endocrine disruptors like PCBs or PBDEs which are common contaminants of human breast milk. Previous reports suggested that 1,25-VD influences mood during the dark season in humans. In addition, we could demonstrate that endocrine disruptors affect sexual differentiation of the brain and subsequent sexually dimorphic behavior in adult rats. Also, circulating sex steroids and 1,25-VD were decreased by exposure. Results indicated that 1,25-VD induced conditioned taste aversion in a dose-dependent manner from 2.5-250 mu g/kg body wt. in male rats. Furthermore, estradiol-induced conditioned taste aversion was decreased in male rats after exposure to dioxin-like and non-dioxin-like PCBs during development. In contrast, testosterone-induced conditioned place preference was elevated in males exposed to a breast milk-like mixture of PCBs. In conclusion, affective stimulus properties of 1,25-VD could be demonstrated in experiments with rats and endocrine disruptors modified the affective response to sex steroids. (supported by grants from State of Baden-Württemberg, Germany, PUG UTOX 97004 and from Federal Environmental Agency, Germany, F+E 29965221/03).

1008 Circadian rhythms in acute and organotypic explants of the hypothalamic suprachiasmatic nucleus of the mouse

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The hypothalamic suprachiasmatic nucleus (SCN) is the primary circadian pacemaker (biological clock) in mammals that controls various rhythms of behaviour, metabolism and physiology. These rhythms with a period length of ~24 hours are synchronized with the external light/dark cycle by retinal photoreception and transmission of light information via the retinohypothalamic tract (RHT). Besides this direct connection with the retina, numerous other brain regions project to the SCN and relay information about the environmental state to the nucleus. This information is integrated by SCN neurons and transmitted to different neuronal targets: endocrine neurons and autonomic neurons in the hypothalamus, as well as to areas outside of the hypothalamus. The role of the SCN in adjusting the activity of target neurons with the clock signal is not clear at present.

In the present study we have developed a technique using multi-microelectrode arrays (MEA) to study in long-duration recordings simultaneously the electrical activity of SCN neurons and target neurons in explants of the hypothalamus of the mouse. Extracellular recordings from acute hypothalamic slices and from organotypic slices cultured on multi-microelectrode arrays mainly exhibited multi-unit activity, mostly without the possibility to discriminate single unit activity. The multi-unit activity was clearly, and reversibly, reduced or completely inhibited by the application of GABA (100 μm) or by tetrodotoxin (TTX, 1 μm). Neurons within the mouse SCN and within regions adjacent to the SCN, including the paraventricular nucleus of the hypothalamus (PVN), expressed circadian rhythms in spontaneous firing rate with periods of 23.5 to 24.5 hours. The circadian rhythm in hypothalamic areas outside of the SCN disappeared after removal of the nucleus showing that the rhythm is generated by SCN neurons. In acute slice preparations the mean activity showed a peak near midday, at CT 7.0 (CT = circadian time), whereas in organotypic slice cultures the time of peak activity was considerably shifted, due to the absence of a retinal input and the lack of a synchronizing stimulus that is able to adjust the rhythm. The time of peak activity was stable across several cycles in both preparations. Treatment of SCN slice preparations at CT 6 with pituitary adenylate cyclase-activating polypeptide (PACAP, 100 nM), one of the principal neurotransmitters of the RHT, caused phase-advances of the rhythm by 2 to 3 hours; treatment with the pineal hormone melatonin (1 nM) at CT 10 elicited phase advances of 4 hours. However, simultaneous application of PACAP and melatonin at CT 10 failed to exert any effect on the circadian phase of the spontaneous firing rate showing a mutual interaction between both phase-shifting agents.

Control of behavior and metabolism by a single transmitter - the role of the transcription factor tubby 1009

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The control of behavior and metabolism is one of the most important aspects of neuronal/neuroendocrine systems. Compounds such as peptide hormones or biogenic amines are used to distribute the information. Among them, adrenergic neurotransmission plays a central role, as it is capable to control behavior and metabolism in a coordinated way. Further downstream in the intracellular signaling cascade, molecules are required that transform a short extracellular signal into a long lasting intracellular response lasting for hours or even for longer. One tempting candidate to fulfill these criteria is the transcription factor tubby. As its name implies, the tubby protein regulates fat metabolism. So it has been shown in knockout-mice that developed a severe obesity resulting in a tub-like body shape. We have cloned and sequenced tub-1 the *Caenorhabditis elegans* homolog of tubby. Gene silencing experiments and heterologous expression of tub-1 showed that it is likely to play an important part in the signaling pathway of the tyramine receptor of *C. elegans*. We could show that the invertebrate homologs of adrenaline, tyramine and octopamine, act through a specific receptor, activate the α-q subunit of the corresponding G-protein, which in turn activates the phospholipase C. Further downstream, we

found a fascinating dichotomy that allows differential regulation of short term effects (behavior via Ca^{2+}-signals) and long term signals (metabolism via the tubby protein). Tyramine and octopamine have two main effects, they increase the movement velocity and they liberate fat stores. The unc-31 mutant that is defective in one part of the Ca^{2+}-pathway, is slow, but develops no obesity. In addition, they are resistant to tyramine treatment, which usually speeds worms up. On the other hand, tubby defective worms move as quick as normal worms, but they are obese. In contrast to the unc-31 worms, they sped up in the presence of octopamine, indicating that this branch of the tyramine/octopamine signaling pathway is not affected in these mutants.

PLC activation releases the tubby protein from the membrane, subsequently followed by its migration into the nucleus, where it activates expression of a certain set of genes. This leads obviously to the fat consumption, enabling long distance travel. On the other hand, tyramine receptor, phospholipase C or tubby defective worms develop a severe obesity. These results indicate that tubby acts as an important transcription factor in the tyramine/octopamine pathway required for the long term effects of this compounds on the metabolic state of the animal.

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1010 Direct molecular consequences of prolonged maternal deprivation on the hypothalmic-pituitary-adrenal axis

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The postnatal development of the hypothalamic-pituitary-adrenal (HPA) axis in rats and mice is characterized by a so-called stress hyporesponsive period (SHRP). Characteristic for this period are low basal corticosterone levels and an inability of limbic (but not systemic) stress to substantially enhance corticosterone release. Recent studies demonstrated, that the quiescence of the stress system during the SHRP is maintained by maternal care. A separation of mother and infants for 24 hours (maternal deprivation) results in an activation of the peripheral stress response. Central markers of the HPA system, such as corticotropin releasing hormone (CRH), the mineralocorticoid receptor (MR) or the glucocorticoid receptor (GR) are down regulated following maternal deprivation. This poster addresses two main questions: (1) What is the molecular mechanism of the activation of the peripheral HPA axis? (2) Are the observed changes in gene expression caused by maternal deprivation or by the elevated circulating corticosterone levels? We therefore investigated the time course of the peripheral and central adaptations that occur during the 24-hour maternal deprivation period in the mouse. We measured corticosterone and ACTH concentrations in blood as well as mRNA expression and protein levels of different central mediators of HPA activity. Our results indicate that the activation of the HPA axis during maternal deprivation might be a consequence of altered sensitivity of NMDA and GABA receptors. Furthermore, our data provide

evidence that some gene expression changes following maternal deprivation are a consequence of the enhanced corticosterone levels, while others change independent of corticosterone activation. In summary, our results shed more light on the molecular mechanism of maternal deprivation, thereby opening up new hypotheses about the cause of long-term adaptations of the HPA axis following early life time trauma.

Effects of a Single Social Defeat on Behavioural and Neuroendocrine Parameters in Rats Bred for Extremes in Anxiety

1011

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The quality and effects of social contact are determined, among others, by the emotionality of the partners. In social interaction experiments, for instance, rats bred for high anxiety-related behaviour (HABs) show more passive social interaction patterns than low anxiety rats (LABs). To get a closer look at the perception and consequences of social defeat, we tested HABs and LABs as intruders in a resident-intruder paradigm. HABs/LABs were exposed to resident attacks two times consecutively to ensure subordinate behaviour. After the second physical attack, the opponents were separated for 30-min by a wired mesh. Thereby intruders were still exposed to the resident's threatening without physical harming. Either 5 or 60 min after exposure, intruders were tested on the elevated plusmaze. To monitor both release of vasopressin (AVP) in the paraventricular nucleus (PVN) and hypothalamic-pituitary-adrenocortical (HPA) axis reactivity, two other sets of intruders were equipped 2 days before the defeat with a microdialysis probe and 4 days before with a venous catheter, respectively. Additional rats were tested for Fos immunoreactivity 2 hours after exposure to the resident compared to handled controls.

Whereas the behaviour of HABs during defeat was characterised mainly by passive behavioural patterns like freezing and ultrasound vocalisation, LABs appeared to be less affected by the resident's attacks, showing more active and explorative behaviour. There was no obvious difference in the resident's behaviour towards either kind of rats. In both HAB and LAB animals the defeat did not affect plusmaze performance. Exposure to a resident effectively stimulated the HPA axis in both groups to a similar extent, but the response was delayed in HABs probably due to a suppression of stress reactions by co-appearance of freezing behaviour and parasympathetic activation. Release of AVP within the PVN was stimulated by defeat only in LABs, whereas HABs showed elevated basal release without a further increase during exposure to residents. Under control conditions, no line-specific difference in Fos protein was measured. After defeat, Fos immunoreactivity in HABs was higher in the anterior hypothalamus and the medial amygdala only compared to LABs.

Thus, while extreme trait anxiety or non-anxiety in HAB and LAB rats, respectively, remained virtually unaffected by a single social defeat, this procedure was an appropriate stimulus to reveal the increased stress vulnerability of HABs. Being genetically predisposed to innate hyper-anxiety, HABs exhibit enhanced passive coping strategies and a delayed HPA axis reactivity during exposure to a resident. These mechanisms are likely to involve differential activation of the anterior hypothalamus and the medial amygdala.

1012 Mice bred for high or low trait anxiety: A new murine model for emotionality

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Mice were selectively bred for extremes in anxiety-related behaviour on the elevated plus-maze in order to establish two lines named high anxiety-related behaviour mice (HAB-M) and low anxiety-related behaviour mice (LAB-M). For this purpose, naive 7-week-old CD1 mice were purchased from Charles River (Sulzfeld, Germany). Males and females displaying the most anxious behaviour and males and females displaying the least anxious behaviour were mated, respectively. After eight generations of outbreeding, we started an inbreeding protocol based on strict sibling-mating.

At present, mice are in the F13 generation, and the HAB-M and LAB-M differ reliably and consistently in their anxiety-related behaviour on the elevated plus-maze with HAB-M showing less entries into the open arms ($p<0.001$) and longer latencies to their first entry into the open arms ($p< 0.001$). While, in the original parental generation, the time spent in the open arms averaged 27.1% in males and 31.7% in females, F13 animals show an impressive divergence, HAB mice spending 0.9% (males) and 1.7% (females) and LAB mice spending 61.5% (males) and 59.4% (females) of the time, respectively, on the open arms ($p<0.0001$). No differences were found between the two lines in their locomotor activity, as being derived from closed-arm entries.

Differences in anxiety-related behaviour between the two breeding lines (F11 generation) were also found in the dark-light avoidance task. In addition to LAB-M and HAB-M, "normal" CD1 control mice (NAB-M) were used for this test being purchased from Charles River (Sulzfeld, Germany). Compared to LAB-M and NAB-M, HAB-M animals spent significantly less time in the lit compartment and made fewer entries into it ($p<0.01$ each). Again, the locomotor behaviour (derived from entries into the dark compartment) was virtually identical in all three groups. This can be interpreted as increased anxious behaviour in HAB mice which is not contaminated with locomotion.

Experiments using rotational activity (stress coping) and ultrasound vocalisation of isolated mice pups will further identify behavioural phenomena associated with trait anxiety. Furthermore, future approaches will focus on neuroendocrine and molecular-genetic correlates in this animal model of human susceptibility to anxiety.

Visuo-spatial attention in the vertical dimension: A TMS study

1013

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Background: Double dissociated neglect patterns in patients indicate that attention to the left, lower and near space may preferentially rely on posterior parietal areas (Rapzcak et al., 1988; Mennemeier et al., 1992) whereas attention to the upper and far space may be more related to the temporo-occipital cortex (Shelton et al., 1990). A recent PET study showed this dissociation for near and far space in normal subjects (Weiss et al., 2000). Using rTMS over parietal and ventral occipital cortex in a horizontal line bisection task, Bjoertomt et al. (2002) could selectively produce neglect to the near or far space, respectively. The goal of this study was to further investigate the neural correlates of the vertical line bisection using TMS and to impair attention to the lower or upper space by either parietal or ventral occipital stimulation.

Methods: Ten normal volunteers had to judge whether a vertical line was correctly prebisected. Short trains of rTMS (10Hz, 5 pulses, intensity 60% of maximum output, Magstim Rapid stimulator, eight-shaped coil) were applied over P3, P4 or ventral occipital cortex (1.5cm dorsal and 2.25cm to the right of the inion for an inion-nasion distance of 35cm). Error rates and reaction times were compared to sham stimulation. In a control experiment reaction time to the simple appearance of the same stimuli was measured.

Results: Stimulation over P3 impaired vertical line bisection. Stimulation over P4 led to a decreased reaction time, whereas reaction time in the control experiment did not change. Stimulation over ventral occipital cortex had no effect on error rate or reaction time.

Conclusion: Only stimulation over P3 impaired vertical line bisection. A differential neglect for either side as described for horizontal lines could not be replicated for vertical lines. Our results indicate a role of the left parietal cortex for visuo-spatial attention in the vertical dimension.

Evidence for a Spherical Geometry of Spatial Color Perception

1014

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Neuronal coding of spatial color perception is most economical performed. The color coding (CC) system provides the information for the elemental color sensations in terms of the amounts of elementary colors, as well as the angular information about their two-

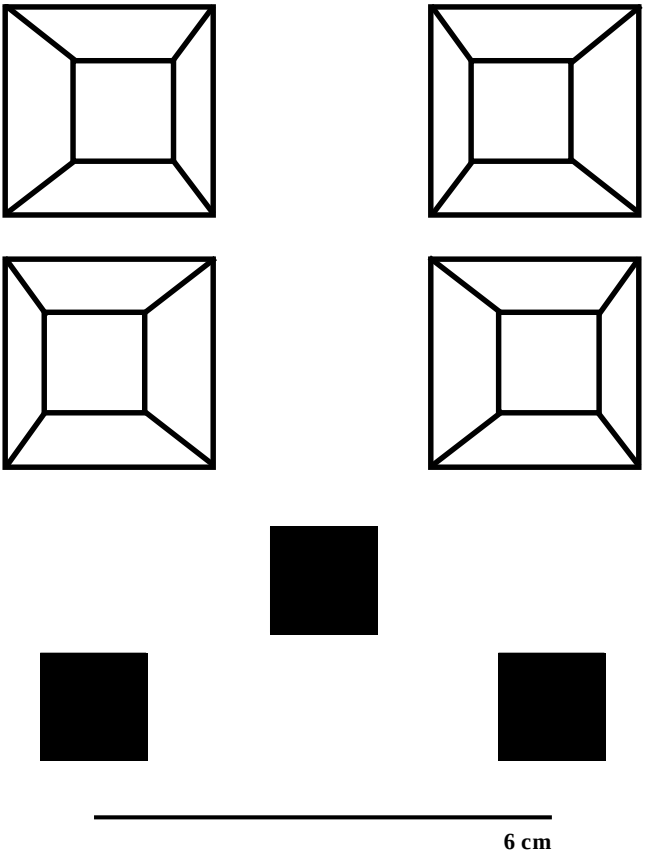

*Figure for abstract **1014**: Evidence for a spherical geometry of spatial color perception: Stereoscopic line-drawings of a truncated pyramid seen from above and below with the left and the right eye (**upper**), and squares seen at two distances (**lower**). The small/black squares are identical in size. 3-D images will be seen, when the left and right images are fused in crossed (or uncrossed) view, i.e. when looked relaxed at the figures on the respective sides from a distance of 30-40 cm with crossed (or uncrossed) eyes. The additional two images will fuse in the center. When converging for 3-D vision, the eyes remain focused on the lines in the paper plane (decoupling of accommodation). Thus, the small/black squares cause identical retinal images. In the 3-D image, the small/black squares appear to be closer to, respectively further away from the observer, compared to the paper plane. Paradoxical, the closer small/black squares appear to be smaller than the more distant small/black squares. The model of spatial color perception (Backhaus, 2001) explains this "phenomenon of enlargement with depth" straightforward as a consequence of the spherical geometry of our visual system (see text).*

dimensional angular positions. The areas of highest resolution in the retinas, the foveas, project to fixed positions in the cortical visual areas (retinotopic mapping). Depth coding (DC) neurons provide the information about the subjective distance of each elemental color sensation, relative to the visual zero-point of the observer. So, neuronal spatial color coding uses a minimum number of neurons. Nevertheless, the provided information is sufficient for conscious spatial color perception with a much higher spatial resolution (hyperacuity) than the arrangements of the photoreceptors in the retinas suggest. Tiny and larger eye movements cause the perceptual areas of highest resolution to move accordingly in the perceptual space. Thus, conscious color perception has to provide highest spatial resolution (hyperacuity) throughout the perceptual space (Backhaus, 2000a,b, 2001a,b).

The model of spatial color perception (see Backhaus, 2001b) predicted a spherical geometry as the simplest realization for steering the angular positions and the radial depths of the elemental color sensations according to the information of the CC and DC neurons. A spherical geometry implies that a color sensation of a certain form and size, e.g. a small black square, must vary in size when the depth varies. Specific experiments have been designed and performed now to test this critical prediction of the model. Line-drawings for stereoscopic images of 3-D objects (e.g. truncated pyramids) have been used to vary exclusively the depth of color square sensations (see Figure).

Results: 1) Although the small/black squares in the drawings have identical sizes, the color square sensations became larger when moving away from the visual zero-point of the observer, and *vice versa*. 2) When converging for 3-D vision, the eyes remain focused on the lines in the paper plane (decoupling of convergence and accommodation). Thus, the squares cause always identical retinal images and the eye lenses do not contribute to the variations in subjective size of the squares. 3) The described "phenomenon of enlargement with depth" confirms the hypothesis about a spherical geometry of spatial color perception.

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Color Discrimination Scales and Elementary Color Scales: Investigations of Nonlinear Relations 1015

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In color content analytical experiments the subjects judge the amounts of elementary colors constituting the color sensations. This type of judgment is related to the experienced colors *per se*, i.e. to conscious color vision. In color discrimination experiments, on

the other hand, the subjective difference between two color stimuli is judged. This type of judgment is supposed to be related to the electrical excitations of the color coding (CC) neurons, i.e. to unconscious neuronal color coding. The gap between the conscious and the unconscious stimulus representations is well described by a black-box that receives input from the CC neurons and with the amounts of the elementary colors as output. The transfer-functions of the black-box are reasonably hypothesized to be piecemeal linear (Backhaus, 1998, 2001). Both types of psychophysical measurements are ongoing performed with identical equipment and the same color stimuli. The measurements of the transfer-functions of the black-box are thus most independent of the graphics card and the color monitor (see Rhoden & Backhaus, 2000). The relations between both the scale types agree to a linear function within maximal error ranges of $^{\pm}$ 8-12%. Nevertheless, within these error ranges the data follow slightly convex and sigmoidal functions (Rhoden & Backhaus, 2001).

Further experimental and theoretical investigations have now been performed to distinguish elementary color specific non-linearities in the causal chain of color perception from other possible influences. 1) The relations between the judged line lengths (one-dimensional case) and area sizes of concentric circles (two-dimensional case) have been measured and compared to the respective objective measures. 2) Possible influences of the light adaptation processes have been estimated, as well as 3) ultimate learning processes during short and long-term experiments. Results: 1) because of the design of the experiments, the measured non-linearities are not due to light adaptation or learning effects; 2) rather, the general form of the curves of elementary color judgments is quite similar to the form of the non-linear curves of the judgments of area size of concentric circles. The non-linear relationships between color discrimination scales and elementary color scales thus appear to be due, at least in part, to the non-linearity of the judgment of elemental amounts in two-dimensional extended sensations.

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Evidence for categorical decision-making in prefrontal cortex – an event-related fMRI study 1016

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Introduction: Single unit recording studies in monkeys have shown that the brain can form a categorical decision by integrating the difference in spike rates from two pools of neurons (“neuron/antineuron” pair, Gold & Shadlen 2001). In a direction-discrimination task (up/down) a decision-variable can be computed by taking the difference between the neural responses of upward-sensitive and downward-sensitive neurons (Gold &

Shadlen 2002). In the present study we tested whether there is evidence for a similar mechanism during human perceptual decision-making. We used a face-house discrimination task be-cause pools of neurons that respond more to faces than to houses and vice-versa can be identified reliably with fMRI in the human brain.

Methods: In a behavioral pilot study with phase scrambled images of faces (face database, MPI for Biological Cybernetics Tuebingen, Germany) and houses, subjects decided whether an image presented on a screen was a face or a house. In the event-related fMRI experiment (n=4, 3T GE Signa, 5-8 runs of 158 volumes, TR 2.0s, 30 trials per condition per run) we used four classes of stimuli. Images of faces and houses with high (percent correct above 95%) and low (corresponding to the 82%-threshold) phase coherence, respectively were shown on a screen for 1s and subjects responded with a button press after a forced delay. To minimize run-to-run variability, noise levels were adjusted according to each subject's actual performance (average phase coherence levels used: faces 91.5%/54.9% (high/low phase coherence), houses 91.5%/46.2%. FMRI data were analyzed using FSL. To define the two pools of neurons representing the evidence for the two alternative categories (face/house), we identified fusiform ('FFA') and parahippocampal areas ('PPA') that responded most to faces and houses, respectively in each subject. We then averaged the BOLD-signal time courses within FFA and PPA, respectively, and computed the absolute value of the difference between the signal in FFA and PPA. In a second step, the resulting time course ('|FFA-PPA|') was used as an additional regressor in the GLM analysis (three-level group analysis using a repeated measures design).

Results & Discussion: The behavioral pilot study showed that images of faces and houses with about 55% phase coherence were identified correctly with 82% probability.Average percent correct rates in the scanner were 96%/74% (high/low phase coherence) for faces and 94%/82% for houses. Response times were 0.57s/0.58s for faces and 0.57s/0.58s for houses. Within the regions that showed a main effect for higher phase coherence independent of category, the following regions covaried significantly with |FFA-PPA|: bilateral middle frontal gyrus (BA8/9), ventromedial prefrontal cortex, posterior cingulate cortex (BA31) as well as fusiform and parahippocampal areas, bilaterally.

Our data are consistent with recent single unit studies in monkeys reporting that abstract decision variables are represented in the dorsolateral prefrontal cortex. This study shows that areas in human prefrontal cortex might indeed form a categorical decision by integrating evidence coming in from lower level areas (e.g. FFA, PPA) over time.

Reaction-time measurments show task-specific adaptations of mental finger representations in profesional pianists 1017

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During piano playing fingers of both hands must be rapidly selected. To study possible limitations of adaptational changes of mental finger representations in highly-trained pianists as compared to non-musicians we measured multiple-choice reaction-times (RTs) during a finger selection task (1).

Instructions were displayed on a monitor and subjects had to press the key corresponding to the selected finger on a hand-shaped 10-key set-up. In experiment I, an image of each hand was displayed and a finger was selected by a visual marker. In experiment II, fingers were selected by displaying the numbers 1 to 5 corresponding to the fingering system of pianists, hands were coded by L/R. In experiment III, fingers were coded by the initials of the fingers (semantic coding).

For pianists, in all 3 experiments, average RTs of all fingers were shorter than those of controls, particularly for experiment II. However, RTs for the middle fingers (MF) were always the longest as compared to the thumb (difference > 100 ms), but differences were smaller in pianists compared to controls. Only pianists in experiment II had largely identical RTs for all fingers. Control experiments testing only 2 fingers revealed no differences under any condition.

The results indicate that practice alters the mental representations mediating the ability to select rapidly a given finger. However, this adaptational advantage is highly task specific, limited to conditions found during piano playing (coding by fingering), but not when a finger is coded either visually or semantically.

(1) Alegria J; Bertelson P (1970) Acta Pycholo

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Recognition of Object Shape during Active Electrolocation in Electric Fish 1018

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Even in complete darkness weakly electric fish can detect, localize, and analyze objects in their environment by employing active electrolocation. They produce electric signals (electric organ discharges, EOD) with an electric organ in their tail and perceive them with cutaneous electroreceptors, which are distributed almost over their entire body surface. Depending on its electrical and spatial properties, an object near a fish projects

an electric image onto its electroreceptive body surface, which is scanned by the electroreceptor array. Neural analysis of the image provides information about object properties such as size, distance, and electrical properties (resistance and capacitance). Here we tested whether the electric fish *Gnathonemus petersii* can perceive an object's three-dimensional shape during active electrolocation. Fish were trained in a two-alternative forced-choice procedure to discriminate between a metal cube and a metal cylinder. Choice of the cube was food-rewarded, choice of the cylinder was mildly punished. Once trained, test trials (no reward or punishment) were interspersed with regular trials. Fish successfully discriminated between the cube and all other objects used. Discrimination performance varied slightly depending on object shape, but was always clearly significant. When instead of a metal cube a plastic cube was offered, fish continued to choose it and not an alternative object, even if this was made out of metal. When the positive object was moved away from the fish, discrimination performance was intact at moderate distance variations but started to deteriorate when distances became much larger. Shape recognition was also invariant of moderate size variations of the object, but deteriorated when cube size was varied strongly. Our results suggest that *G. petersii* can recognize an object's shape independently of object material, and prefer shape over material for object categorization. Shape recognition is also tolerant of small variations in object distance and size.

1019 Duration but not Level of Intense Inducer Tones Affect the Loudness Reduction of Subsequent Weaker Tones

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A moderate-level tone drops in loudness, when it is preceded by a more intense tone. This effect has been called loudness recalibration by Marks (J. Exp. Psych. Hum. Perc. and Perf. 20, 382-396, 1994) and induced loudness reduction (ILR) by Scharf et al. (JASA, 112, 807-810, 2002). The amount of ILR (i.e., the decrease in the weaker or test tone's loudness level) was measured as a function of the duration and level of the stronger inducer tone. The loudness of a 500-Hz test tone at 60 or 70 dB SPL was matched to the loudness of a 2500-Hz variable-level comparison tone in a two-alternative forced choice procedure. Test and comparison tones lasted 200 ms. Results for twelve listeners showed that the amount of ILR depended on the duration of the inducer tone, but not on its level; 5-ms recalibration tones, whether at 80, 95, or 110 dB SPL, yielded only 3 to 4 dB of ILR. Long inducer tones at 80 (200 and 500 ms) and 95 dB SPL (200 ms only) yielded 7 to 11 dB of ILR, again with no apparent effect from the level of the inducer tone, but with more ILR for a 70-dB than for a 60-dB test tone. Although the 5-ms inducer tones at 95 dB SPL were about as loud as the 200-ms inducer tones at 80 dB SPL, they yielded much less loudness reduction. A follow-up experiment addressed the question if 5-ms inducer tones induce little ILR because they were so brief in absolute terms or because they were so brief relative to the 200-ms test tones. The test and comparison tones were now shortened to 5 ms. Results for twelve normal listeners showed that inducer tones at 80 dB SPL--whether 5 or 200 ms long--yielded the same average amounts of ILR for the 5-ms test tones: 4 dB for 60-dB test tones and 9 dB for 70-dB

test tones. Taken together, these experiments suggest that (1) ILR is governed neither by the loudness nor by the sound pressure level of the recalibration tone and (2) the amount of ILR is small when the inducer tone is briefer than the test tone. [Supported by NIH/NIDCD R01DC02241]

Discrimination of Visual Numerosities by Monkeys: Object Tracking versus Analog Magnitude Representations. 1020

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Recently, monkeys have been introduced as model organisms to study the neural correlate of numerical competence on the single neuron level. The precise nature of the behavioral process encoding numerical information, however, remained speculative.

We analyzed the behavioral discrimination performance of two rhesus monkeys for numerosities 1 to 7 in a delayed match to sample task. The monkeys showed similar discrimination performance irrespective of the exact physical appearance of the stimuli, confirming that performance was based on numerical information alone. The performance declined smoothly with larger numerosities. Increasing the numerical distance by a constant fraction (Weber-fraction) resulted in similar discrimination performance. Response times increased only mildly up to four items before they reached a plateau. No proportional increase in the number of eye movements with increasing numbers of items was observed, indicating that the animals did not serially scan individual items in the displays before deciding about a numerical match. Both findings argue for parallel assessment of numerical information.

We conclude that the monkeys behavior is not compatible with non-numerical processes of number discrimination (subitizing, object-file representations), but can best be explained by numerical analog magnitude representations. The behavioral results are compared to single-cell recordings from the prefrontal cortex in the same subjects, as well as fMRI studies in humans.

Chromatic priming across the vertical meridian in normal and hemianopic subjects 1021

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In fields of cortical blindness caused by destruction or denervation of the primary visual cortex, several residual visual functions can be demonstrated even though the patients remain unaware of the stimuli (Blindsight). To learn whether the brief presentation of a chromatic stimulus in the blind field would modulate the response time to a chromatic target in the normal field, and whether any effect depends on the colours of the stimuli,

we tested six hemianopes and eight normal observers with a PC-driven VDU, using the eye with the defect in the temporal hemifield. A blue or yellow 2° target appeared on the grey background in the (normal) nasal hemifield at one of three equiprobable positions 3.5° from the fixation dot. Subjects were asked to press the one of the three response buttons assigned to the target's spatial position as fast as possible when the blue 'Go' target appeared. The yellow target was equiluminant to the blue and required no response ('No-Go'). Target presentation was terminated by the subject's response, or after 1,500 ms. Prime stimuli (200ms, 6° diameter) preceded the targets by 150 ms. They appeared at only one position which, in each patient, was adjusted to the individual defect but was always in the blind temporal field. On 80% of 'Go' target presentations, a congruent blue prime was presented; the remaining 20% were equally often preceded by an incongruent yellow prime, or by a blank. Similarly, the rarer 'No-Go' (yellow) targets were preceded by a yellow (80%), blue, or blank (each 10%) prime.

Priming effects were analysed by non-parametric tests for individual subjects by comparing the cumulative reaction time distribution ('Vincentization') obtained in the different experimental conditions. The results revealed a colour-specific positive effect of the prime in six control subjects; in the remaining two, the blue and yellow primes indiscriminately decreased the RTs. Interestingly, there was no unspecific effect of this kind in the patients. Instead, two showed no effect, two showed a significant effect of the incongruent (yellow) rather than the congruent (blue) prime, and in two the specific effect seen in 75% of controls was observed in that responses to validly primed targets were significantly faster than those to invalidly or unprimed ones. As the colour of the unseen prime mattered in four out of our six patients, the retinofugal pathways that survive the lesion and subserve the hemianopic field must be providing chromatic information that can be used to accelerate motor responses to seen stimuli, as has been reported for normal observers. The present paradigm makes it possible to study these processes without forcing the patients to guess about unseen stimulus properties, and reveals colour-specific interactions between the normal and blind fields.

1022 Successful treatment of tinnitus by transcranial magnetic stimulation - a case report

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A 35-year-old, otherwise healthy patient come to our hospital in a reactive depressive state after tympanoplastic surgery due to Tinnitus. In spite of conventional treatment, his dysphoric mood state did not improve, and we thus decided to utilize transcranial magnetic stimulation. We employed our standard procedure: Dantec magnet stimulator at 110 % intensity of the motor threshold with a circular coil. We stimulated a spot in the left prefrontal cortex 5 cm. anterior and parasagital of the spot on the precentral gyrus that evoked a motor potential. After 10 treatment days (15 Hertz, 1000 impulses/day), there was unmistakable clinical and subjective improvement. The Tinnitus and depressive symptoms had both almost completely remitted.

Lightness Constancy: Shades are compensated in perception, scattering light not 1023

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Luminance of three-dimensional achromatic objects under given illumination depends on remittance of the material, shading and scattering light (mutual illumination of opposed surfaces). We studied perception of lightness of six flat surfaces, three exposed to direct illumination, three in the shade in opposite position, thus allowing mutual illumination of neighboring pairs. Six achromatic cardboards forming a logarithmic scale of remittance (step factor = 1.2) were used. Direct illumination increased the luminance of the cardboards by a factor of two with respect to those in the shade. The luminance sequence of the cardboards varied with their positions in light and shade. Altogether there are 720 permutations of the sequence of the six cardboards. For any of the 720 permutations selected for the experiments, the subjects tried to report the lightness sequence correctly in spite of the different luminance sequences. The difference between the empirical lightness sequence reported by the subjects and the physically measured sequence according to remittance was taken as the measure of lightness constancy. We used Kendall´s rank correlation coefficient τ, which is $\tau_R=1$ if the two above sequences are identical. We also calculated τ_L from the empirical and the luminance sequences. If the subjects were unable to perceive the lightness, they would report the luminance sequence with $\tau_L=1$ and $\tau_R<<1$. Tests with 13 subjects on 30 different permutations yielded $\tau_R>\tau_L$ (with exception of control experiments in which the sequence according to lightness and luminance happened to be the same physically, yielding $\tau_R=\tau_L=1$ correctly). Thus, lightness constancy of three-dimensional objects under asymmetric illumination as reported since more than 100 years (summary e.g. in A. L. Gilchrist: Lightness, Brightness, and Transparency, 1994) has been confirmed quantitatively with our novel method. In addition, a quantitative result concerning mutual illumination can be derived from our data. Mutual illumination of cardboards can change the luminance sequence as a consequence of neighborhood. From a light cardboard under direct illumination more light is scattered to and reflected by its dark neighbor in the shaded position, than from the same cardboards in exchanged position. We calculated the degree of lightness constancy separately for those cases, in which the luminance sequence was not changed by mutual illumination, and those were the difference with the lightness sequence was increased and decreased. Separate calculations of these groups revealed that the effect of local scattering light makes it more difficult to detect the correct lightness. Mutual illuminations increasing the difficulty resulted in less constancy (τ_R smaller), whereas mutual illumination resulting in greater similarity of the lightness and luminance sequences (thus reducing the difficulty) yielded better constancy (τ_R greater). The effect of light scattered locally at three-dimensional objects is not taken into account in the process leading to lightness constancy of perception.

1024 Osmotic minipumps as a stress-free method of chronic imipramine administration: Behavioral study in C57Bl6/N mice

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Chronic treatment is a part of many models of human disorders and of pharmacological studies that often causes the undesirable effects on tested animals. Osmotic minipums (OMP) is a recent invention that was designed, on one hand, to overcome stress effects of daily drug injections, and on another hand, to assure a preciseness of delivered dosage that other stress-free methods, like per os administration, could not provide. However, the potential drawback of OMP employment in behavioral studies can be the physical disturbance of animals' movement, especially when OMPs are used for long treatment and in small animals, like mice. Therefore the aim of our work was to study whether subcutaneously implanted OMP designed for 4-weeks-long drug delivery (ALZET 2004; 3.0 cm length, 2.1 g weight) would affect locomotion and exploratory behavior, that is sensitive to stress in C57BL/6N mice (3.5 month old, average body weight 29.3g).

The osmotic minipumps (ALZET 2004) were filled with vehicle or imipramine solution of concentration that assured 20 mg/kg/day dosage of drug delivery and were implanted subcutaneously to male CB57BL/6 mice. Ten days after surgery OMP-implanted mice were analyzed in a battery of behavioral tests, consisting of open field, novel cage and Porsolt tests. As a control, not-treated group of mice and group of mice, receiving imipramine in the same dose with drinking water were taken.

In the open field test the OMP/imipramine and OMP/vehicle -implanted mice showed similar total distance moved and mean velocity as compared to mice, receiving imipramine with water and to the non-treated controls. Similarly, in Porsolt test the latency of floating and the duration of floating did not differ between three groups of mice. OMP-implanted mice swam normally, when scored by the visual observation. In a novel cage exploratory test, documented to be sensitive to the influences of stress, OMP/imipramine and OMP/vehicle -implanted mice displayed similar numbers of rearings in comparison to mice receiving imipramine with drinking water and to the non-treated controls.

These data show that nor 10-days long imipramine administration nor OMP-implantation affect locomotion and exploratory behavior, sensitive to stress. Notably, relatively large OMPs designed for 4-weeks long drug administration were found to not disturb spontaneous locomotion and swimming in Porsolt test in mice. Thus, the application of OMPs provides a stress-free methods of drug administration in mice while achieving its stable concentrations in blood, that can have high implication for psychopharmacological studies in animal models.

Modulation of human direction discrimination by cognitive demands

1025

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Previous work on direction discrimination of moving visual stimuli often tested the performance of human subjects only with respect to a single or a small number of reference directions. In addition, test and reference stimuli usually were moving along a linear trajectory. In our study we investigated in greater detail how direction discrimination is modulated by the frontoparallel stimulus direction and whether this performance is influenced by different motion trajectories and/or additional cognitive demands.

In the first series of experiments, we measured psychophysically in fixating human subjects their perceived direction of motion (PDM) of a random dot pattern (RDP), using a modified version of the Transformed Up Down method (Levitt 1971). Computer generated visual stimuli (25 degree aperture) and the fixation target were presented on a monitor (100 Hz) at a viewing distance of 57 cm. Subjects viewed two RDPs in sequential order. The first RDP moved either on a linear or on a circular pathway, the second RDP always moved on a linear pathway. Subjects had to indicate whether the movement direction of the second stimulus was rotated clockwise or counterclockwise compared to the final movement direction of the first stimulus (2 AFC). Each subject's (n=6) performance was tested at 24 different, equally distributed directions, giving a resolution of 15 degrees in the frontoparallel plane. In a second experiment we presented an auditory stimulus simultaneously with the RDP (moving on a circular pathway) in the first stimulus interval. As auditory stimulus we used a series of clicks whose frequency was modulated sinusoidally. Subjects had to indicate whether the movement direction of the second stimulus (linear translation) was rotated clockwise or counterclockwise compared to that of the first RDP at the moment of the peak-frequency of the auditory stimulus (i.e. direction discrimination with an additional cognitive demand). Again we measured the PDM for the frontoparallel plane in steps of 15 degrees.

For translational motion all subjects' mean PDM did not differ significantly from the reference direction (mean difference: -0.03 deg). For circular motion the mean PDM was -11.19 deg, i.e. the PDM was shifted 11.19 deg against the circular motion direction. In addition, a strong modulation of the shift across the different reference directions was observed. For the simultaneous visual and auditory stimulation the mean PDM was shifted 25.45 deg against the circular motion direction. This larger difference is probably due to the visual-auditory task resulting in a higher cognitive demand. The modulatory pattern of the subjects' performance in this bi-modal task was very similar to the one observed in the visual only task.

Our results indicate that the ability to discriminate the direction of a moving visual stimulus is strongly influenced by the motion trajectory and additional cognitive demands.

1026 Which components of language are sufficient to activate the hand motor system?

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Speaking, covert reading of and listening to sentences reliably activate the hand motor system. The origin of this cross-system activation is still not thoroughly understood. Speech comprises semantic, grammatical, and prosodic features. The purpose of the present investigation was to examine whether presentation of the prosodic component of sentences in isolation suffices to activate the bodily action system.

We systematically varied the auditory input and measured hand motor cortex excitability through motor evoked potentials (MEP) induced by transcranial magnetic stimulation (TMS) in 12 subjects with left-sided language dominance. Stimuli consisted of a baseline condition with no acoustic stimulation, bitonal sounds (tones differed in an octave, same duration as normal sentences), flat prosodic contours with minimal pitch variation (same duration as the normal sentences), variable prosodic contours without semantic and syntactic information (matched for prosodic contours with the normal sentences), and sentences including all three components of language.

Listening to tones or monotonous bisyllabic intonations did not excite the hand motor cortex. Both listening to variable prosodic contours and to sentences bilaterally activated the hand motor cortex. For the left motor cortex, there was no difference in the extent of activation through variable prosodic contours and sentences. The right motor cortex, however, responded significantly stronger during the perception of variable prosodic contours as compared to the sentences. Furthermore, the pitch variability of the sentences correlated selectively with the size of the MEP amplitudes after right motor cortex stimulation.

The findings replicate our previous findings of a tightly connected neural system for manual and linguistic gestures. The present study shows that the prosodic aspect of sentences is sufficient to activate the bodily action-perception network. Future studies should examine which other isolated aspects of language/speech can trigger the motor cortex activation. The result confirms the assumption that language did not evolve as a separate module, but within a more domain-general action-perception network for coding gestural information.

1027 Psychoacoustic thresholds in the Mongolian gerbil (*Meriones unguiculatus*): A comparison of methods.

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'Psychoacoustic threshold' is generally used to describe the limits of perception in the auditory system. In many animal studies psychophysical functions are determined using

the method of constant stimuli (MCS) to calculate a threshold value. Although these methods are accurate and reliable, they are also time consuming, because a lot of data are collected far away from the threshold level. If repeated threshold data need to be collected in an individual to determine the effects of certain manipulations faster algorithms are desirable. We directly compared the MCS, that is well established for the gerbil (for details of the method see Hamann et al., Hear Res. 171, 82-95, 2002) with adaptive procedures (AP) by measuring thresholds for 2kHz- and 10kHz-tone stimuli and broad-band noise pulses in 6 gerbils. AP thresholds were determined using either 2dB-steps (AP2S) or 3dB-steps (AP3S), respectively. A session started well above the expected threshold. As long as an animal responded to the signal the level was reduced in 2 or 3 dB steps. After a trial where an animal did not respond the signal level was increased by 6dB (reversal). Subsequently the level was decreased again and a total of 6 reversals was recorded. The mean of the detected and missed levels preceding the last 4 reversals was taken as threshold value. About 25% of trials were used as sham trials and only runs with less than 20% false alarm rate were included. A final threshold was calculated from 2 valid sessions with threshold difference not greater than 3dB. The following table lists average thresholds (in dB SPL) and standard deviation (in brackets) for the 3 test signals determined by MCS, AP3S and AP2S, respectively.

2kHz			10kHz			broad-band noise		
MCS	AP3S	AP2S	MCS	AP3S	AP2S	MCS	AP3S	AP2S
0.4	0.1	4.8	16.1	18.2	18.0	12.5	13.6	13.6
3.4	4.8	7.8	2.7	3.6	3.5	0.8	5.5	3.1

Thresholds measured by the 3 procedures were very similar. However, the average test-time to obtain a threshold by MCS was 177min (ranging from 53 to 403min), while the average test time was only 50min (ranging from 15 to 181min) for AP. We saw no systematic difference in this small sample of animals between the 2dB and the 3dB procedure. These preliminary data show that AP procedures are feasible in the gerbil and they save time compared to MCS.

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Temporal integration in the Mongolian Gerbil 1028

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The detection thresholds of pure tones decrease if the duration of the test tone is increased. This ability of the auditory system to integrate acoustic energy over time is generally referred to as temporal integration.

The Mongolian Gerbil (*Meriones unguiculatus*) has become a suitable animal model in auditory research because of its similarities to the human auditory system. In this study gerbils were trained in a GO/NOGO-paradigm to report the detection of a pure tone stimulus of 2 kHz. Signal durations were 5, 10 and 800ms. The test signals were presented according to the method of constant stimuli.

The animals show a mean increase in threshold of 7,7dB (± 4,2; n=12) and 12,9dB (± 4,4; n=10) if duration is decreased from 800 to 10ms and 5ms, respectively. The mean increase of threshold between 10 and 5ms was 6,4dB (±2,7; n=10). There was a significant correlation between the observed threshold shift of the 10ms-pulse and the absolute threshold of the 800ms-pulse (Pearson, r^2=0,45; p=0,016; n=12). No significant correlation was found between the threshold shift of the 5ms-pulse to the 800ms or the 10ms threshold (Pearson, r^2=0,24 and r^2=0,002; n.s., respectively; n=10). In 3 animals the threshold of the long 800ms pulse was elevated by about 10-33dB compared to normal hearing animals. These animals showed the lowest threshold shift in response to the 10ms of only 3,3dB (± 1,5; n=3) in contrast to the sensitive animals that showed 9,1dB (± 3,8; n=9). These findings are in good agreements with previous data on humans, where impaired listeners also showed a reduced temporal integration. This shows that the Mongolian Gerbil is a suitable model for studying temporal integration. Further, data will be compared to other studies on temporal integration in mammals and birds.

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1029 Perceptual and working memory impairments in first-episode schizophreniform psychosis and established schizophrenia

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The delayed-matching-to-sample (DMTS) paradigm from the Cambridge Neuropsychological Test Automated Battery is especially suitable for investigating the contribution of perceptual and memory processes relevant to object working memory. The task contains immediate and delayed recognition trials (0, 4 and 12 seconds delay) as well as a simultaneous matching (SM) control condition.

Using this task, we acquired data from 55 first-episode schizophreniform psychosis (FE), 50 established schizophrenia (SZ) patients and 56 gender-matched healthy controls (CTL). Preliminary analyses of the error data revealed a skewed distribution, which was subsequently modelled using negative binominal regression, covarying for age and premorbid IQ. This model revealed task difficulty as a significant predictor for error score of the delay dependent tasks (0, 4 and 12 seconds delay), with patients performing worse than CTLs. After controlling for perceptual performance (SM component) performance deficits in patients could still be found. SM performance was shown to be a significant predictor of delayed matching task performance.

In conclusion, while patients had greater difficulty on the task even at simultaneous matching, indicative of attentional or perceptual problems, this did not explain the impairments observed during the delayed matching conditions. A delay dependent impairment on this task is apparent as soon as the stimuli are no longer accessible in the environment. This may result from a deficit in the ability to internally represent the complex visual patterns.

Differential effects of tactile coactivation on spatial and frequency discrimination: Psychophysics and FMRI in humans

1030

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A few hours of tactile coactivation induce significant reorganization of the paw representation in primary somatosensory cortex (SI) of adult rats. Changes are characterized by an enlargement of the cortical areas representing the stimulated skin sites and of the corresponding receptive fields and by increased neuronal response durations (1). In humans this coactivation paradigm led to comparable reorganization of the primary somatosensory cortex, paralleled by improvement of spatial discrimination performance (2-4). Here we demonstrate that different somatosensory discrimination tasks are differentially effected by this kind of passively induced cortical reorganization.

For coactivation, separated receptive fields on the right index finger (digit 2) were simultaneously stimulated for 3 hours as described in (2). Pre and post coactivation, we performed psychophysical tests of spatial and temporal discrimination performance, accompanied by FMRI mappings of the somatosensory cortical representations of digit 2 of the right (test) and left (control) hand with a 1.5T Siemens scanner. For testing spatial discrimination performance we used gratings of different spacings which orientations on the finger tips had to be discriminated by the subjects. For temporal discrimination tests vibrotactile stimuli of 500 ms duration with frequencies between 25 Hz and 35 Hz were applied and subjects had to decide if the frequencies of the test stimuli were higher or lower than that of a 30 Hz reference stimulus.

We found that the somatosensory cortical representation of the finger stimulated during coactivation was selectively enlarged as compared to the representation of the control finger. These plastic changes were accompanied by differential effects on spatial and temporal discrimination performance. Thresholds for spacing of gratings in the orientation discrimination task were decreased, indicative of improved performance. On the other hand, frequency discrimination thresholds increased. Both effects were found for the test but not for the control finger. Combined, our results reveal the possibility to induce specific perceptual learning effects with pure passive tactile stimulation without attention or reinforcement.

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(4) Godde et al. (2003) Neuroreport, in press. Supported by Volkswagen St

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1031 The influence of individual conductances on spike timing for inputs with dominant frequencies

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Neurons *in vitro* and *in vivo* can respond to repetitive presentation of the same stimulus with precisely timed and reproducable spikes. More reliable (i.e. more precise and reproducable) spike timing potentially enables a neuron to transmit information at a higher rate. The reliability of responses to a given stimulus depends on the statistics of the input (such as variance and frequency content) as well as on the biophysical properties of the neuron. A change in the effective densities of ion channels of a neuron can lead to a different reliability of the responses to the same stimulus. Such a change may be mediated by neuromodulators on a fast time scale and thus allow a cell to quickly adapt to new input statistics.

Many neurons *in vivo* have been found to participate in network oscillations and receive rhythmic input in different frequency bands. We therefore focus on stimuli that exhibit dominant frequencies and investigate how modulation of ion channel densities affects spike timing reliability in response to such stimuli.

We employ a conductance-based model of a pyramidal cell to analyze the influence of Hodgkin-Huxley type sodium, potassium, and leak channels, a slow potassium current, and a persistent sodium current on reliability. We show that several intrinsic membrane currents modulate the frequency preference of model cells, as measured by their reliability in response to sinusoidal current injection. Spike timing reliability is quantified by a measure based on pair-wise correlations between trials.

We find that slow potassium currents are especially effective in modulating the frequency preference within a range of 5-70 Hz and therefore may be a prime target of neuromodulators. The increase in reliability by optimal tuning of potassium channels with slow kinetics is not only observed for pure sinusoidal inputs, but also for more realistic rhythm-like stimulus waveforms in the Theta (4-12 Hz) and Gamma frequency (30 - 70 Hz) bands. Dynamic-clamp experiments in rat cortical slices confirm the theoretical prediction.

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Itô Calculus Approach to the Distribution of ISI and Response-Stimulus Correlation

1032

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Major neural learning mechanisms like spike timing dependant plasticity, synaptic redistribution and synaptic scaling depend on - and change - the neurons inter-spike intervall distribution, as well as the distribution of time differences between stimulus and the neurons response. The first is identical to the first passage time (FPT) of the membrane potential, whereas the latter can be expressed as a function of the neurons mean firing rate and the response-stimulus correlation (in experimental contexts also called the reverse correlation).

In order to gain a deeper understanding into how these major learning mechanisms work we have a closer look at the underlying processes which shape the above two distributions. We try to come up with an analytical expression for these important distribution densities - the latter of which has up to now only been computed numerically - aiming for a trade-off between biological realism and mathematical tractability.

We use a leaky-integrate-and-fire neuron with reversal potentials, in which synaptic inputs are collectively modeled as an Ornstein-Uhlenbeck process driving the neuron. Neglecting the synaptic time constants the membrane behaviour satisfies a linear stochastic differential equation (SDE). Using Itô Calculus and the method of the integrating factor this SDE can be solved, and the moments of the solution can be calculated.

These moments are used to approximate the probability of threshold crossings and thus give an approximate, but analytic solution to the first passage time problem (FTP), the distribution of inter-spike intervalls (ISI) given the input. There is no known exact solution to this problem, even for the simple leaky integrate-and-fire neuron with additive white noise.

Simulations show that the membrane potential exhibits time symmetry. If the threshold is neglected it is possible to approximate the response-stimulus correlation for the subthreshold regime. Starting with the potential at the threshold value - where it has just produced a response spike - the flow of the potential can be followed backwards in time. From this an explicit expression can be derived which states the expected value of the driving stimulus at a given time before the occuring response spike.

All our results are compared to numerical simulations of the corresponding stochastic processes to demonstrate the quality of the approximations. The incorporation of more biologically realistic parameters (like a more realistic noise model) is subject of current investigation.

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1033 Modeling perceptual learning in the primary visual cortex: Passive unsupervised or active reinforcement-based sensory reorganization?

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It is known that visual perception improves with experience. Up to now, however, is it not clear whether this improved performance is due to a change in the representation in the visual cortex, an improved read-out of an otherwise unchanged representation or a mixture of both. Recently it has been shown [1] that after training some visually responsive neurons in monkey prefrontal cortex reflect the membership of stimuli to learned categories and may thus be part of the neuronal correlate of perceptual learning. On the other hand, it is also tempting to assume that not only read-out processes are adapted during learning, but also the representation itself, because studies from auditory and somatosensory cortex have shown behavior-related reorganizations of sensory representations. Indeed, it was found that improved performance in an orientation discrimination task is correlated with local reorganizations of circuits in primary visual cortex in the sense of enhancing the representational accuracy of selected orientation domains by means of, e. g., sharpening orientation tuning curves [2] or changing the preferred orientations of neurons [3]. The observed changes, however, leave open the question whether information from the environment is utilized to adjust the representation in the visual cortex or if the reorganization is done in an unsupervised manner.

We set up a model of a population code representation of orientation information in the primary visual cortex and simulate the experimental protocol used in psychophysical orientation discrimination tasks. We model the two learning scenarios of (i) adapting the representation in an unsupervised manner and (ii) adapting the representation using the reinforcements from the environment. Both learning scenarios are modeled as stochastic approximations which utilize gradient information in order to minimize an objective function. In the first case the 'goodness' of a representation is measured via the mean squared error of the stimulus reconstruction. In the second case it is measured by the expected classification error when discriminating two classes of oriented stimuli. Obviously, in the second case top-down class information is needed to reorganize the low-level representation which could be provided, e. g., via feedback projections. In the first case local bottom-up information is sufficient.

We find that the representation resulting from the unsupervised adaptation simply reflects the frequency of the observed oriented stimuli whereas the reinforcement-based adaptation leads to enhancing the representation of the boundary between the stimulus classes which should be discriminated. Based on our simulation results we can make predictions for the time-course of the reorganization during learning which may allow to experimentally distinguish between the two scenarios.

[1] Freedman et al., Science, 2001
[2] Schoups et al., Nature, 2001
[3] Ghose et al, J Neurophysiol, 2002

Optimal information transmission in a parallel array of integrate-and-fire neurons

1034

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It is well established, that noise can improve the transmission of weak signals in threshold elements. This effect, called stochastic resonance, has been extensively studied in the context of single neurons. Metabolic considerations and neurophysiological measurements indicate that biological neural systems prefer information transmission via many parallel low intensity channels, compared to few high intensity ones. Thus the information transmission properties of parallel summing arrays of neurons are of substantial interest, and it was recently shown, that the optimal noise level, which maximizes the information transmission over the array, depends on the statistical properties of the input signal and on the number of array elements (Stocks, Phys. Rev. E, 63.04114, 2001, p.1-4). Thus adaptation of single neurons to the optimal noise level, would require the knowledge of the array size. But this is information about the global architecture of the system which is not - in an obvious way - available locally at the single cell.

We consider a parallel summing array of leaky integrate-and-fire neurons in which the input to each neuron is the sum of a common aperiodic Gaussian stimulus and additive white Gaussian noise. We show that the information rate is maximized at an optimal noise level and that this optimal noise level increases with the number of neurons in the array sub-logarithmically. Furthermore, the information rate curves are very flat at the maximum, which indicates, that the amount of transmitted information does not degrade in a dramatic way for a relative broad region around the optimal noise level. Therefore, we show that a local learning rule performs almost as good as a global one for sufficiently large number of neurons in the array ($N>100$). Note that this would be the case for the size of typical cell assemblies in cortex.

On the other hand, these findings change dramatically if we take metabolic considerations into account. Information transmission in nervous systems is metabolically expensive, especially the generation of action potentials consumes a huge amount of energy. Thus to ensure energy efficient coding we postulate that it is advantageous for neurons to maximize the ratio between transmitted information and cost. We assume that the average energetic cost per unit time is a function of the activity of the neurons in the array and a fixed amount of basis cost. Numerical simulations demonstrate that only for the supra-threshold region the optimal noise level depends on the number of neurons in the array, whereas it does not depend on the array size ($N>10$) for sub-threshold signals. Thus in the latter case the single neurons can use locally available information to adjust to the optimal noise level, even if they do not know the global architecture of the entire system.

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1035 Contrast adaptation by adjusting neurotransmitter release probability in a hypercolumn model of visual cortex

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It has been proposed (see [1]) that contrast adaptation is at least partly due to the change of neurotransmitter release probability at the thalamocortical synapses. The model there consists of one layer of recurrently connected excitatory cortical neurons corresponding to one orientation column in cortex that receives geniculate input from sinusoidally modulated Poisson processes with identical phase. Afferent and recurrent connections are endowed with depressing synapses [2]. The adaptation to different levels of contrast is achieved via slowly changing the probability of neurotransmitter release at the feed-forward synapses in a way that the mutual information between input and output firing rate is maximized. Our goal now is to translate the above ideas into a biologically more relevant framework. Here we extend this model to a full orientation hypercolumn of coupled excitatory and inhibitory conductance-based integrate and fire neurons. The LGN input to this hypercolumn is modeled as a two-dimensional grid of sinusoidally modulated Poisson processes. Each cortical neuron has a certain orientation prefference and has connections coming from the LGN according to its receptive field (ON regions) as well as recurrent connections to its neighbours in orientation space. We use a narrow distribution of excitatory and a broader distribution of inhibitory connections to achieve contrast invariant orientation tuning. Each afferent synaptic connection is explicitly modelled as depressing, each recurrent connection as non-depressing, depressing or facilitating synapse. To derive a learning rule we again make the hypothesis that the mutual information between a cortical neurons output firing rate and its geniculate input firing should be maximal. For the output firing rate we use an approximation of the contrast response function of the model. We show that the experimental findings of contrast adaptation ([3]) can be reproduced by regulating neurotransmitter release at the afferent but not recurrent connections. Specifically different temporal components of the membrane potential and spike response at the optimal and near optimal orientations in our model show qualitatively the same behavior as in the experiments. We also observe a phase advance relative to the driving stimulus with adaptation, that is with reduction in release probability, as has also been experimentally observed. We conclude that our biologically more realistic model reproduces all findings of the previous model and shows that the hypothesis of adaptation of probability of neurotransmitter release is able to explain important experimental observations related to contrast adaptation.

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[3] Carandini et al. (1997). Science 276, p. 949-952

A model for the reaching reflex of an infant 1036

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In this presentation a model that imitates a behavior which occurs during a learning step of an infant will be examined. The model is based on a unsupervised learning algorithm that builds up on the hebbian learning rule and on the rescorla-wagner theory.

The behavior during the learning step of an infant which the model will imitate is well analysed in the Developmental Psychology and known under the name reaching reflex. An infant learns within it's first sixth months of living how to reach successfully for an object. Therefore the learning task for the model is to construct a neuronal structure which activates muscles in relation to a visual input.

The model operates in a simulated environment and it consists of a neuronal structure. The structure gets its main input through 40x20 visual neurons. In relation to the visual input the model has to activate motoric neurons to move a virtual arm consisting of two limbs. The neuronal net operates spike based. Therefore the model has to excite the motoric neurons with a specific spike pattern to reach successfully. The network consists of two different structures which create at different time the arm movement. In the beginning the movement is made through an unmodifiable neuronal feedforward networkstructure which creates spike patterns for the motoric neurons in dependency of environmental regions in which an object is. The resulting spike patterns which occur in the net are used as training examples for the learning algorithm to build up a new link structure which is the second structure that is used later from the model to create the arm movement.

Spoken abstractly the learning algorithm extracts from the spike patterns causal relations between different spikes and successful reaching. It determines which spikes are relevant and which are not for success. The links are used to encode this information. They store a weighted spike pattern with temporal information. The weights are used to express the relevance of different spikes within a pattern. With this links it is possible to represent complex relations. It's for example possible to express that reaching is often successful if a visual neuron n1 and n2 creates spikes 10 ms before a motoric neuron m1 creates one. The task of the learning algorithm is to build up the links and to weight the encoded spikes with the associated timings. One main reason for using this new kind of link is that when a classical recurrent network structure would be used every causal relation needed to be expressed through an own subnetwork structure and the number of neurons in the net would explode.

The movement of the arm with these links works in that way that the net tries to recreate the causality expressing spike patterns with the help of activation propagation through the links. The resulting movement is better than the movement through the initial structure.

1037 Metabolic aspects of information transmission in the noisy leaky integrate-and-fire neuron model.

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Including considerations about the metabolic cost of neural activity has a long and successful tradition in theoretical and experimental studies of neural information processing. Metabolic cost in neurons is strongly related to spiking activity and thus tightly connected to information transmission. High intensity signals are reliable but metabolically expensive, low intensity signals are more economic, but are unreliable and introduce a probabilistic component to neural coding. So, for optimal information processing reliability and metabolic cost have to be balanced. We investigate the influence of noise on the input-output relation of a generic model neuron and thus the influence of noise on this trade-off. The neuron is modeled as an Ornstein-Uhlenbeck process with an upper absorbing boundary. The drift term corresponds to the signal input, the diffusion term resembles the membrane potential fluctuations (noise). Given drift and diffusion the response can be calculated in terms of the moments of the interspike interval distribution (ISI). We assume that there is a fixed amount of metabolic cost per unit time as a baseline. Costs due to informations transmission are considered to be some power of the response spike count within a given time interval. It is assumed that noise is either for free or adds to the metabolic baseline cost. At this point we do not make assumptions about the source of the noise. We assume that neural coding is based on the spike count within a certain time interval. In order to quantify the amount of information transmitted by the neuron, we employ the three following measures: (i) the difference in spike count, (ii) the discriminability, i.e. the difference in spike count divided by the standard deviation of the noise, and (iii) the mutual information, what is a measure of how much information about the input distribution is available from the output distribution. Given the above measures we first investigate the information transmission normalized by the metabolic cost for Gaussian input distributions. The input mean ranges from very low to high intensity values. We demonstrate that sub-threshold signals - signals that are so weak that they would not elicit a response without membrane potential fluctuations - should be employed for information transmission, once metabolic costs are considered. So a significant amount of unreliability due to weak signals might be acceptable in neural systems to save metabolic costs.

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Odor stimulation induces changes of correlation between glomeruli in the antennal lobe of Honeybee 1038

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In the primary olfactory center on the honeybee, the antennal lobe, odors are represented as patterns of active neural clusters, called glomeruli. These glomeruli receive input from the chemosensory receptors and are synaptically connected to projection neurons and local interneurons. In the absence of odor stimulation the glomeruli show fluctuating low level spontaneous activity. We investigate the statistics of these spontaneous activities and their changes after odor stimulation. We find that after a single odor presentation the spontaneous activity is modified: some glomeruli are more active whereas other glomeruli are less active. Activity increases predominantly in those glomeruli that belong to the activity pattern induced by the odor stimulation shortly before. This effect was found in 10 out of 15 preparations. Moreover, a single odor presentation also induces changes of pairwise correlation between spontaneously active glomeruli: the correlation between some pairs of glomeruli increases whereas it decreases between other pairs. These changes are significant within a 95% confidence interval and again occur predominantly among glomeruli that belong to the activity pattern induced by the odor stimulation shortly before.

In different bees the same odor induces different changes of spontaneous activity and of pairwise correlation between spontaneous active glomeruli activities. Despite such variability it is in general possible to infer the kind of odor just from the changes of correlation between pairwise activities during spontaneous activity. Since odor induces activity in multiple glomeruli, Hebbian plasticity may lead to reorganize the network in such a way that spontaneous activity will reflect the formerly active glomeruli. This interpretation is in line with our findings that a combination of statistics and linear algebra allows to reconstruct the odor specific pattern from the change of correlation between glomeruli in over one third of the bees.

Such a Hebbian plasticity could be the mechanism of olfactory sensory memory in the antennal lobe.

1039 A neural field model for planning of saccadic eye movements: Dependency of saccadic decision making on target separation and fixation condition

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Selecting one from many objects in the visual field and directing the gaze to it by a saccadic eye movement is a fundamental behavior of primates. The investigation of this behavior by means of the double-target paradigm reveals a dependency of the saccade metrics on target separation and saccade latency. For large target separations the saccade is directed to one of the targets (target selection), for small separations the saccade lands between the targets (target averaging), and for intermediate separations a speed-accuracy tradeoff is observed: saccades are target-averaging for short latencies, but target-selecting for long latencies [1,2]. It has been proposed that this behavior is caused by the successive action of two different neural systems [1].

We demonstrate that the psychophysically observed separation and latency dependent decision making concerning the plan for the impending saccade is a basic property of a neural field model with simple structure. We therefore propose that this behavior is generically generated in the brain structures concerned with saccade planning, and that no complicated neural mechanisms are needed.

A central feature of our model is a pattern of recurrent interaction with short-range excitation and long-range inhibition ("Mexican-hat" interaction). Kopecz and Schöner previously showed for the double-target paradigm that a neural field model captures the dependence of target averaging and selection on target separation due to its Mexican-hat interaction pattern [3,4]. We developed a neurally more realistic extension of the Kopecz-Schöner model with separate layers for excitatory and inhibitory neurons for each neural field. Our model additionally captures the dependency of saccadic decision on latency. The model consists of two fields. In the planning field a saccadic motor plan in form of a single peak of activity (resembling population coded saccadic motor activity in the Superior Colliculus) is generated from the target stimuli. The planning activity is coupled into the fixation field where it competes with fixation activity evoked by a foveal stimulus. When the fixation activity has been suppressed, the elapsed time (saccade latency) and the center of mass of activity (saccade endpoint) are read out. We employed gap, step and overlap fixation conditions which served to produce short and long latencies. The model property which brings about the speed-accuracy behavior is the inhibitory feedback coupling causing the inhibition to lag behind excitation. In the time course of the planning dynamics there is an excess of excitation initially, followed by a balanced state. Therefore, inhibitory competition of stimulus evoked activities leading to target selection takes place at larger target separations for short latencies than for long latencies.

Our model qualitatively reproduces the saccadic endpoint distributions of the psychophysical experiment. Moreover, a prediction is made that could be tested psychophysically: For certain target separations and latencies, both target selection and target averaging can occur. This is indicated by trimodal endpoint distributions.

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Activity patterns in neural layers are enhanced by disordered connectivity

1040

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Spatial activity patterns like target waves or spirals can emanate in two-dimensional layers of excitable media. Examples in biology include visual hallucinations, which are believed to be synchronized waves in the striate cortex [1,2], and spiral waves in heart tissue which can be induced by successive stimulation at one single point [3].

We are interested in the role of stochastic connections in facilitating pattern formation. To address this question we applied disorder models to a twodimensional network of leaky integrate-and-fire neurons.

In the deterministic case the connection matrix of each neuron has a Gaussian distance dependency of the coupling weights multiplicated with a proportionality factor I_0. After introducing a *disorder parameter* - a measure for the stochasticity in the model - by randomly changing the deterministic Gaussian connectivity matrix we are interested in the minimal factor I_0 which gives rise to temporally stable activity in the twodimensional layer of neurons.

In the first model the deterministic Gaussian connection strength between two neurons is the mean of a γ-distribution from which the coupling weight is drawn at random. The variance of the distribution plays the role of our disorder parameter.

In the second, stronger disorder model a certain number of coupling weights are interchanged. The corresponding disorder parameter is the ratio P of interchanged weights and all weights.

The results of the simulations show that $I_{0,\min}$ necessary to get temporally stable activity decreases with increasing disorder parameter. That is, in both models disorder in the connectivity yields a facilitated formation of temporally stable patterns. Moreover, for the second model, an optimal exchange ratio P exists.

Our results may be relevant in a neural layer like the visual cortex where increasing disorder by reorganization of the neural connections helps producing temporally stable activity. An example could be drug induced visual hallucinations [1].

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1041 Building Representations Spike by Spike

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Brains enable animals and humans to rapidly recognize and classify sensory scenes using spikes as signals, that come from the receptors and from within the brain. It appears that on average one spike per neuron and processing step is sufficient to achieve a good performance. This is particularly astonishing when considering the apparent randomness of cortical spike trains.

Here we focus on the question how reliable representations can be built from noisy input spike patterns containig only a small number of spikes per input neuron.

We present a novel framework for neural computation which utilizes synaptic nonlinearities (like the NMDA receptor) to extract most of the information contained in each individual spike. This is demonstrated in a network that iteratively builds a sparse and noise-robust representation of a noisy spike input. The weights in this model are adapted on-line using an unsupervised Hebbian rule that can be shown to optimize the representation. We first test the performance of our network, using different learning rules and reconstruction paradigms, by building representations of simple binary functions such as XOR, AND, and OR. The computational power of our framework in this well-defined task is contrasted to the performance of classical approaches of building representations, which often make use of an off-line batch learning, and a non-iterative, noise-free reconstruction method.

In the next step, we use our rather general ansatz to tackle much more sophisticated problems: when interpreted as a model for the cortex, and trained with natural images, our framework reproduces many properties of visual cortical cells including simple cell receptive fields, complex cell responses, and non-classical receptive fields. We also investigate, how the structure of long-range connections, as measured within the visual cortex, may emerge during learning by extracting correlations between inputs and sequences of inputs to our model.

Our results indicate that the visual cortex is optimally adapted for rapidly building representations of visual scenes from about one spike per input neuron. The model predicts characteristic temporal evolutions of the intracellular responses, depending on the spatial structure of the visual input, which can be tested experimentally.

A robust method for the estimation of tuning curves and the encoding accuracy of neural populations 1042

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An important issue in the Neurosciences is a quantitative characterization of the relation between sensory stimuli presented to an animal and their representation in the nervous system. In the recent past, statistical estimation theory has been employed to determine the encoding accuracy of neural populations given responses to natural and artificial stimuli.

However, electrophysiological data are typically very noisy. In this contribution, we show that the same set of experimental data can lead to dramatically different conclusions with respect to the achievable encoding accuracy depending on how the tuning curve is constructed from the recorded data. In particular, the widely used method based on the least squares approximation can perform very badly; this is mostly due to the fact that it is sensitive to outliers.

We suggest a rank-based method for the estimation of tuning curves which yields more stable results and therefore more reliable computations of the performance of neural populations in encoding sensory stimuli. The method is applied to both artificially generated data and recordings from rat primary primary visual cortex.

Dynamics of Random Networks: Current-Based vs Conductance-Based Synapses 1043

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The dynamics of sparsely connected random networks of Integrate-and-Fire (I&F) neurons connected by current-based synapses have been shown[1] to exhibit different network activity states, depending on the external input firing rate nu_{ext} and excitation/inhibition balance g: synchronous-regular (SR), synchronous-irregular (SI), asynchronous-regular (AR), and asynchronous-irregular (AI). Here, we performed simulations of random networks of I&F-neurons connected by current-based synapses (I) and by conductance-based synapses (II). In both cases, we studied the network activity dynamics and the single-neuron firing patterns under systematic variation of the parameters nu_{ext} and g.

We found that both network models exhibited the AI-state over a considerable region of the parameter space. In Network II, the AI state makes a transition to the SR-state state already for low nu_{ext}, only weakly dependent on g. For small networks we observed a clear influence of network size, which saturates for networks in the order of 10,000 neurons or more. We are currently exploring the influence of synaptic time constants,

heterogeneity, more realistic (Hodgkin-Huxley) neuron models, and more realistic connectivity rules[2].

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1044 Dual Coding Principle: A Unifying Concept in Interaural Time Difference Localization

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Time differences occurring between both ears are an important cue for animal and man to localise a sound source. The neuronal representation of interaural time differences (ITDs) in the medial superior olive of small mammals seems to deviate strongly from the traditional Jeffress model. Based on a computational model for the development of synaptic couplings, we show how spike-timing-dependent plasticity of both excitatory *and* inhibitory synapses elucidates the existence of precise arrival of afferent currents and, hence, of tuning curves as observed in experiment, with a maximum of activity at ITDs leading at the contralateral ear. The model also accounts for the experimentally found dependence of best ITD upon stimulus frequency and explains experimental data providing strong evidence for experience-dependent inhibitory plasticity. In order to study the neuronal representation of ITDs, we present a neuronal realization of an idea dating back to von Békésy (1930). It is capable of unifying two as yet contradictory hypotheses brought up by Jeffress and Mc Alpine et al. in that stimulus ITD is represented by neuronal population activity in two alternative ways simultaneously, a principle we name *dual coding*. Depending on external parameters, such as the spectral composition of the sound, the head size of the animal, and even the azimuthal position of the sound source, either one or the other representation can be more reliable. We explain the ontogenetic development of an architecture that embodies the dual coding principal through a non-local mechanism of synaptic plasticity called axon-mediated synaptic learning.

1045 Precise Spike Patterns in Complex Neural Networks

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The precise timing of action potentials is believed to play an important role for the representation of stimuli and internal events. Thus, a central question is to identify the dynamical origins of spike patterns. Recently, it has been shown that periodic spike sequences may be attractors of the dynamics of neural network models with global

inhibition [1]. Here, we employ a model of pulse-coupled neurons [2,3] to study the emergence of such spike patterns in complex networks (cf. [4]) with inhomogeneous connection strengths. We demonstrate the existence and stability of periodic temporal spike patterns. If the inhomogeneity is too large, the patterns are replaced by an asynchronous state. Furthermore, we show how to predict spike patterns from a given configuration of synaptic strengths. Reversely, we present an approach to prescribe a coupling architecture in order to obtain a desired pattern.

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What determines the timing of a spike? 1046

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Neurons communicate by sending and receiving action potentials (spikes). These action potentials are generated in the soma, where under natural conditions the membrane potential (MP) changes constantly due to the time dependent inputs received by the neuron. While it is often assumed that a spike is emitted when the MP crosses a threshold voltage V_{thres}, neurons *in vivo* and biological sophisticated conductance based models behave differently. There, however, a thorough analysis of the underlying spike evoking mechanism is very involved.

As a first approach to understand the consequences of more complex spike evoking mechanisms, we analyzed a simple model in which a spike is emitted when $V(t)$ crosses a threshold curve in the V-dV/dt plane. The MP $V(t)$ is modelled as a stationary Gaussian stochastic process. Such a process is completely characterized by its mean value $\langle V \rangle$ and its correlation function $C(\tau)$. For concreteness we assumed the threshold curve to be linear, i.e. $(dV/dt)_{thres}=aV+b$. With these assumptions we analytically calculated the population averaged output rate without further approximations. It turned out that the firing rate is completly determined by the parameters a and b of the threshold function and four parameters of the MP: the mean MP $\langle V \rangle$, its variance $\sigma(V)^2$, the variance of its velocity $\sigma(dV/dt)^2$ and the variance of its acceleration $\sigma(dV^2/dt^2)^2$.

To assess the validity of our model, we compared our results with MP time series from neurons measured *in vivo*, as well as with numerically obtained time series from conductance based model neurons. Our results indicate that both assumptions, the Gaussian process as well as the linear firing threshold are justified. Take together, our study suggests that the firing rate crucially depends on the higher order statistics of the MP.

1047 A Monte Carlo Simulation of intracellular signal propagation in an autocatalytic reaction

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Signal transduction often leads to a regulation of the transcription pattern of a cell. The very mechanism by which a signal translocates from the cell's surface to the nucleus remains in many cases unclear. Wave propagation of autocatalytic reactions has recently been reported to be a potent mechanism, which can propagate a signal over a long distance in an efficient and fast way. Castiglione et al [1] analyze the dynamics of such a variant of intracellular signal propagation in an idealized medium, where free diffusion of a particle in all different directions may occur.

We extend this study to a more realistic framework using an inhomogeneous medium containing randomly distributed obstacles, which constrict the directions, in which a particle can diffuse. We perform a Monte Carlo simulation to determine the slowing down of transport due to intracellular obstacles.

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1048 Homeostatic Adaptation in Neural Systems

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The concept of homeostasis introduced by W. B. Cannon in the 1920s has recently been revived in a number of contexts ranging from the regulation of synaptic efficacies to behavioral and social phenomena. Referring to studies in control theory, we present general learning mechanisms based on the homeostatic principle and discuss applications to self-structuring of neural networks and to motor control problems. Our approach is based on the idea of dual control, i.e. the execution of a control task and the simultaneous tracking of parameter changes. We study dynamical systems consisting of a controller-predictor unit interacting with an environment and showing behaviors which can be analytically understood in the case of a single unit. Several such units form small-scale networks where the environment of a unit consists of other units of the same kind. Each unit is adapted based on a functional of the difference between actual or predicted input states and set-values. Small differences can be considered as self-generated rewards which introduces a relation of homeostatic learning and continuous-time reinforcement learning. An assembly of many interacting controller-predictor units can be considered as a working example of Cannon's principle, which we, emphasizing its dynamical aspect, want to refer to as homeokinesis. At a higher level of description, similar units are used in a phenomenological model for the perceptual basis of movement coordination (cf. F. Mechsner et al., Nature 414 (2001) 69), which naturally follows from the homeostatic principle: Motoric parameters are adapted relying on predic-

tability of self-controlled stimuli which is enhanced for symmetric input. In a more general setting we show that elementary behaviors can arise in a self-organized manner such as to reflect specific properties of the sensor-motor loop, to favor smooth trajectories, and to allow for quick re-adaptation within a set of elementary behaviors.

Network dynamics and functional connectivity from fMRI 1049

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Data from fMRI experiments contains information about functional connectivity among brain areas, which is, however, superimposed to stimulus-related activity, vegetative processes, and disturbances. We assume that structural properties of functional connectivity can be revealed by analyzing correlations between measured activity time courses of individual pixels after appropriate preprocessing. More specifically, we define correlation graphs with pixels as vertices by including a connection between two vertices only if the corresponding correlation exceeds some threshold. For a variable threshold we obtain a family of graphs which become more and more sparse as the threshold rises. In addition to global properties such as mean connectivity, substructures of the graphs are of interest such as strongly connected (i.e. highly correlated) subgraphs. By comparison with an equivalent family of random graphs, i.e. a family which reproduces certain properties of the data graphs, a threshold value can be determined that renders the retrieved subgraphs as most striking.

We further compare the frequency and significance of correlated subgraphs of thresholded-correlation graphs to those in dynamic correlation networks. The latter are defined analogously to an artificial recurrent neural network with the data correlations serving as connectivity strengths. After initialization by an activity configuration the activity in the network evolves until a stationary activity distribution is reached, which is repeated for several initializations. Limit configurations reproduce the connectivity components of the above data graphs or retrieve to a good approximation the fully connected subgraphs (cliques), for respectively suitable values of the "neural thresholds". Beyond these two limiting cases, we study subgraphs of intermediate connectivity in the dynamic approach, which are hard to identify in graphs.

If instead of symmetric correlations time-delayed correlations are used, the connectivity of the dynamic network is asymmetric, such that convergence of the activity towards a limit configuration is no longer guaranteed. Instead, an on-going activity can be observed in the network, which is comparable to the measured fMRI activity. Finally, we consider adaptation rules for the artificial network which tend to synchronize the measured and reconstructed activities and discuss implications for large-scale models of the brain.

1050 Motion perception during pursuit eye movements: A neural network study

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One of the basic problems the visual system faces during eye movements is that the information coming from the eyes reflects both objects and eye movements. During pursuit of a moving target, for example, the retinal image of the target is nearly stable, yet the visual system is able to compensate for the eye movements such that the pursued object is perceived as moving. We are usually unaware of this compensation process, although in pathological cases where this function is impaired, patients are unable to construct a stable percept of the environment. Eye movement compensation is a complex process involving integration of visual information with an internal eye movement signal. In a previous study, we introduced a physiologically based neural network model of motion processing during pursuit eye movements. The model consists of three layers of computational units, simulating neurons in relevant cortical areas. The units in the third layer, like some neurons in area middle-superior-temporal (MST), receive, in addition to the visual input, an extra-retinal signal related to eye movements. The population response of these units can be interpreted in terms of perceived movement. In the present study our model was used to analyze perceptual phenomena and address basic questions related to pursuit eye movements. We simulated the perceived path of a stationary or circularly moving object, during pursuit of a second circularly moving object. We show that model responses match a variety of related psychophysical findings. By analyzing the model simulations, the perceptual phenomena are explained by the underlying neuronal mechanisms, thus helping to fill the gap between single-cell and system levels. Finally, the present study supports the hypothesis suggesting a significant participation of the eye-movement related signal in the perceptual process during pursuit.

1051 Stick insect walking pattern generation - a 3D neuro-mechanical simulation study

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Insect walking relies on a complex interaction between the environment, body segments, muscles and the nervous system. For the stick insect, in particular, previous investigations have highlighted the role of specific sensory signals in the timing of activity of central neural networks driving the individual leg joints. Three aspects have been identified as important: (i) movement signals in intrajoint control; (ii) velocity and position signals in interjoint control and (iii) load/strain signals in interjoint co-ordination. Recent advances in our detailed knowledge of the underlying neurophysiological mechanisms encouraged us to utilize computer simulations to test the consequences of the emerging hypotheses. The simulations incorporate models of the stick insect body bio-

mechanics, muscle properties and the neural control system; and make the direct study of the interaction between these subsystems possible.

The 3D biomechanical model was developed based on the species Carausius morosus. Morphological data, including sizes, weights, and centre of gravity of each body segment, were taken either from published work (Cruse, 1976) or collected in our laboratories. The main body is represented as a single segment, while each leg is simulated as three segments: coxa, femur and tibia. The tarsal claws are currently not represented explicitly. Muscles are included in the form of torque generators at the joints. The simulation is set up so that realistic length-tension and velocity-tension characteristics can be used. The present muscle model in our simulations is rather simple, based on implicit parameters calculated from results of Storrer (1976; PhD Thesis; Kaiserslautern).

The neural control system has been encapsulated in a special module in the simulator. This module receives information resembling true sensory signals, such as ground contact, joint angles, and load. Based on experimentally known neural responses, the module then calculates the muscle activation levels which enter the muscle model. At present, the neural control system uses two principles: (i) Height control realized by a graded negative feedback from the position of the coxa-trochanteral joint (Curse et al. 1993); (ii) Switch timing in a finite-state system of three bistable mechanisms where sensory signals trigger transitions between the internal states. The timing controller is based on data from recent physiological experiments investigating how specific sensory signals affect the timing of individual joint controllers (e.g. Bässler, 1988; Hess & Büschges, 1999; Akay et al. 2001; Bucher et al. 2003).

The first aim of the simulation was to test the sufficiency of current knowledge to explain the generation of the co-ordinated stepping pattern of the stick insect middle leg. Except for height control, sensory control of motor activity is implemented by discrete switching depending on sensory thresholds. The current minimal configuration of the simulated model is able to reproduce the co-ordinated stepping movement of the middle leg. As a further step towards a realistic simulation of the complete stick insect walking system a simulation with active movement of both middle legs was set up. With hind and front legs stiff and friction free, the middle legs alone are able to generate forward propulsion of the body.

Do fly motion-sensitive neurons receive spike-triggered or graded synaptic input? 1052

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In many invertebrate and vertebrate sensory systems neurons are found that show graded membrane potential changes in their axon terminals that regulate transmitter release continuously. In other neurons only spikes are transmitted to the output region of the cell.

In the visual system of the fly some motion-sensitive interneurons produce spikes while others respond to stimuli with graded de- and hyperpolarizations. There are also motion-sensitive neurons with a mixed response mode, i.e. graded potential changes superimposed by rapid spike-like depolarizations. Neurons with all response modes receive synaptic input from the same population of presynaptic neurons. Although the motion-sensitive interneurons (e.g. H1 and HS) are well characterized it is not known yet whether their presynaptic elements transmit information spike-mediated or in a graded fashion. Based on a conductance-based model of an interneuron that receives input from either spiking or non-spiking model neurons, we analyze which features of the synaptic transfer function lead to realistic postsynaptic responses.

Graded presynaptic potentials were shown at several synapses to induce changes via a shallow transfer function that is centered around the resting potential of the presynaptic neuron, thereby transmitting the whole range of continuous changes. For spiking neurons, in contrast, the transfer function has been often found to be steep and to have a high threshold. As a consequence, subthreshold membrane potential fluctuations are prevented from being transmitted.

In the first step of the analysis, only spontaneous activity (as it is produced by fly visual interneurons in the absence of visual stimulation) was simulated. The synaptic transfer function was chosen to induce postsynaptic responses with temporal and spectral statistical properties matching experimental data.

Surprisingly, our model results indicate that spontaneous activity of fly motion-sensitive interneurons can be reproduced neither by standard synaptic transmission of spikes nor by classical continuous transmitter release. Instead, for both input types the same transfer function - a steep sigmoid centered around the presynaptic resting potential - was found to be optimal. Shallow transfer functions lead to a variance of the postsynaptic potentials that is considerably lower than it was found experimentally. For transfer functions with a high threshold the power spectrum of the postsynaptic potentials does not fit experimental data. Therefore we predict on the basis of the spontaneous activity that a mixture between the properties of conventional spike-mediated and continuous synaptic transmission might be used by fly motion sensitive neurons.

In the next step, we will expand our model analysis to motion-induced activity as it can be observed experimentally during visual stimulation. This analysis will be particularly interesting because linear transmission between presynaptic neurons and the motion-sensitive neuron has been assumed implicitly so far in all models of the fly's visual motion pathway.

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1053 A dynamic computational model of goal-directed perception

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Recent experiments suggest that vision might be influenced by internal goals prior to recognition. I present here a novel computational model that simulates the systems dynamics to explain how higher cognitive processes influence early visual processing.

Notably, within this model visual attention is not explicitly implemented, but rather emerges as a natural consequence of competitive interactions between brain regions (specifically, in this case, V4, IT, FEF and PFC).

It is proposed that visual areas first acquire information about the scene by processing visual stimuli in a parallel bottom-up manner and acquire a more detailed knowledge about an object of interest by feedback. Activity from the ventral pathway then converges onto premotor neurons to enforce behavioral decisions (in this case FEF movement cells), which in turn are fed back onto cells in the ventral pathway. This reentrant activity has the effect of implementing a spatially organized gain control of the item of interest. We implemented aspects of the above mentioned areas by a mean field approach.

This model successfully replicates previously collected data from single-cell recordings in IT and V4 from a macaque visual search task (Chelazzi, Duncan, Miller, Desimone, J. Neurophysiol. 1998; Chelazzi et al., Cereb Cortex 2001). It was suggested that competitive effects among IT cells are biased by prefrontal areas. Our simulation results offer support for an extended explanation in which the movement cells in the FEF or the occulomotor system (FEF, LIP, SC) in general facilitate the processing of the target object in the ventral pathway due to a reentry signal (Hamker, Soc Neurosci Abstr 2001). It predicts that the late target discrimination around 150ms after scene onset is based on reentry from premotor neurons. The model is also consistent with the temporal dynamics of other recordings in FEF (Schall, Philos Trans R Soc Lond B Biol Sci 2002). Moreover, by representing visual features as populations of active cells, whose activity is dynamically modulated in a parallel manner, this model offers an efficient solution to the problem of target localization within a cluttered visual display (e.g. natural scenes).

This model goes beyond current theories of attention in neuroscience which have taken a local view. They still assume that attention is a source that is doing something, e.g. attention biases competitive interactions or attention increases the gain. We suggest a framework that acts on the network level. The distributed mechanisms within the perception-action network evoke attention. Attention itself is better described as a result of such mechanisms like gain control by feedback, cooperation, and competition - an aspect of parallel processing for recognition, planning and action preparation. The brain tries to resolve interference in order to connect high level task descriptions or actions with low level scene descriptions.

Functional consequences of presynaptic inhibition in an oscillatory network – a simulation study 1054

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Presynaptic inhibition of synaptic terminals can be a powerful mean of regulating synaptic activity and motor output. In the stomatogastric nervous system of the crab, presynaptic inhibition seems to play an important role in the central pattern generators that drive the pyloric and the gastric mill rhythms (Coleman et al., 1995, Nature 387:502-505). The projection neuron MCN1 modulates the pyloric rhythm and excites the two gastric mill half-center cells Int1 and LG (Blitz et. al., 1999, J. Neurosci.

19:6774-6783). LG's excitation is mediated by a fast electrical and a slow chemical EPSP. At the same time, the MCN1 axon receives presynaptic inhibition from LG, which causes a decrease in transmitter release on the pyloric and the gastric mill rhythm. Consequently, the timing of the gastric mill rhythm and the speed of the pyloric rhythm are strongly influenced by LG activity (Bartos & Nusbaum, 1997, J. Neurosci. 17:2247-2256). The pyloric cycle frequency should thus depend on the strength of the presynaptic inhibition and LG firing frequency.

We are using the network simulator *madSim* to model the neuronal network underlying the generation of the pyloric and gastric mill rhythm of *Cancer pagurus* and to test the impact of presynaptic inhibition on the timing of the gastric mill rhythm and on gastro-pyloric circuit interaction. *MadSim* is a newly developed simulation environment for realistic neural network modeling which adapts and extends the kernel of the unix-based BIOSIM tool (Bergdoll & Koch, 1995, Neurocomputing 8:93-112) to WINDOWS. It is capable of creating networks up to several hundreds of neurons. Here, neurons according to the SWIM model (Ekeberg et al., 1991; Biol. Cybern. 65, 81-90) were used. For the pyloric rhythm, a network was constructed which consisted of 5 classes of neurons and produced a triphasic pattern with a cycle period of 1.15s. For the gastric mill rhythm, the core pattern generating neurons LG and Int1 were modeled. MCN1 excited both networks and Int1 received inhibition from the pyloric pacemaker neurons PD. Although almost all morphological cell properties are disregarded in *madSim*, we succeeded in modeling the physiological effects of such functional units. Presynaptic inhibition of MCN1, for example, was achieved by connecting the MCN1 axon with an electrical synapse to a nonspiking neuron with graded transmitter release. This 'terminal' then received inhibition from LG which decreased action potential amplitude. To model the different time courses of MCN1's electrical and chemical synapses LG was provided with a fast and a slow dendritic region.

We are currently investigating the effects of changes in LG firing frequency and of changes in the strength of the presynaptic inhibition from LG to Int1 on the pyloric cycle frequency and on the LG burst offset.

1055 MadSim - a tool for simulating biological neuronal networks

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BIOSIM (Bergdoll & Koch, 1995; Neurocomputing 8: 93-112), a simulation environment for neuronal networks was originally designed for UNIX systems. It critically depends on outdated MOTIF libraries. The less powerful PC version (1992) is not suitable for research studies. We thus converted the UNIX kernel of BIOSIM to WINDOWS and developed a new graphical user interface, *madSim*, which also extends the original BIOSIM functions. In *madSim*, electrical synapses can pass depolarizing and hyperpolarizing currents, while electrical synapses in BIOSIM block hyperpolarizing currents. Further features include the selective export of simulation results in ASCII, easy comparison of simulation results, downward compatibility with BIOSIM and export and import of single neurons and selected parts of a network. Furthermore, graphical parameters like color, size and name of a neuron or the appearance of a result window can be modified. Besides the use of zoom and cursor functions for data analysis, the

current result waveform can be stored as a reference for comparison. Additionally, a convenient access to neuron and result window properties with the possibility to alter several parameters of a set of selected items simultaneously is provided. A straightforward structure of the simulation files opens the possibility for manual editing and further processing.

To test the functional integrity of *madSim*, we compared the results of BIOSIM and *madSim* by simulating an oscillating network with recurrent inhibition based on the SWIM model (Ekeberg et al., 1991; Biol. Cybern. 65: 81-90). The comparison of the soma potentials of both simulations showed no differences. In contrast, the modification of the electrical synapses had a great impact on the simulation results. Since *madSim* and BIOSIM differ in the processing of electrical synapses, *madSim* was equipped with the possibility to use the BIOSIM electrical synapses. A comparison of identical networks with electrical synapses that passed either only depolarizing currents (BIOSIM synapse) or depolarizing and hyperpolarizing currents (*madSim* synapse) revealed that the type of synapse not only had a strong influence on network activity but also on the activity of single neurons. In general, *madSim* synapses increased the stability of oscillatory networks.

Up to now only the SWIM model has been implemented in *madSim*. We are currently adding further types of neuron models like the Golowasch-Buchholtz model (Buchholtz et al., 1992; J. Neurophysiol. 67: 332-340). For easy and fast network development an option for parameter iteration in a user-specified range and stepwidth is planned. At present, we are using *madSim* to build the neuronal networks underlying the femur-tibia control system of the stick insect, the flight control system of the locust and the gastric mill system of the crab.

Binding and Synchronization in Reciprocally Connected Cortical Areas 1056

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We developed a model of two reciprocally connected visual areas using spiking neurons to investigate scene segmentation and binding of distributed representations in the visual system. The topographically organized peripheral area P is modeled similar to the orientation modules of the primary visual cortex, while the central area C is modeled as an associative memory representing stimulus objects according to Hebbian learning.

We observed two different modes of oscillatory activity resulting from the bidirectional interaction between the two areas: a state of slow activity with only locally synchronized spike activity in the peripheral area P, and a state of fast activity where synchronization is more far reaching and the cells in the lower area are coordinated with one of the patterns in the associative area. Both activity states switch dynamically on a scale of several hundred milliseconds. Presenting a superposition of several stimulus objects neurons representing the same object are simultaneously in the fast state and also switch simultaneously to the slow state due to habitation.

Further simulation results suggest that our model is compatible with phenomenons observed in various neurophysiological experiments such as stimulus-dependent synchronization of fast oscillations, synchronization on different time scales, ongoing activity, two-state-fluctuations of membrane potential, and attention-dependent neural activity.

Extrapolating the results from our simulations we suggest a more global model for binding of distributed representations (neural assemblies) in the brain. The so called binding problem (BP) occurs in three forms: 1.Binding between neurons coding co-occuring features, 2.Binding across different feature dimensions (such as form and color), and 3.Dynamic binding (e.g. role assignment) relating different assemblies where possibly fast synaptic plasticity is required. A solution to BP1 is demonstrated by our current model. BP2 can be solved by a straight-forward extension. Even for BP3 our model suggests a solution without requiring fast synaptic plasticity.

1057 Periodicity Pitch Detection and Sound Separation with Spiking Neural Networks

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We implemented a biologically motivated neural model (based on Langners pitch processing model) with spiking neurons for extracting pitch from sound and tested it first with noisy AM input and second when noise is added to the neuron potential. We then tried some modifications of the neuronal model based either on current biological findings or on theoretical thoughts, and compared these results to the results from the original model. One goal of the modifications is to either eliminate or suppress the comb-filter characteristics from the original model, which can be achieved by modifying some parts from the original neural model, or by introducing appropriate inhibitory connections.

We then tested the model with various vowels spoken with different pitches. The results showed that the model is able to extract both the pitch and the formant frequencies of the vowels, and that it is practically important to find a solution for the comb-filter behavior.

Another purpose for using the model is sound separation: Since phase locked spikes can be used to separete sounds from different sources by binding frequency channels with synchronous spikes, it is important to understand how the model should be exactly built to get spikes as phase synchronous as possible. This binding problem is actually much easier to solve on the level of periodicity pitch detection neurons because these neurons typically spike only once every period of the envelope (and not every period of the carrier frequency).

Two-states membrane potential fluctuations driven by weak pairwise correlations 1058

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Physiological experiments demonstrate the existence of weak pairwise correlations of neuronal activity in mammalian cortex. The functional implications of this correlated activity are hotly debated. Nevertheless, it is generally considered a widespread feature of cortical dynamics. In recent years another line of research has attracted great interest, the observation of a bimodal distribution of the membrane potential defining up- and a down-states. Here we show that the latter phenomenon is a natural consequence of the former. This link explains the observed increase of the power during the up-states in the ?- frequency band and an increase in the standard deviation and fraction of time spent in the depolarized state. Furthermore, bringing together these two aspects of the cortical dynamic under a unified view allows to directly relate results obtained in one context to the other, suggesting a new experimental paradigm: specific intracellular analysis can be a powerful too to reveal properties of the input correlation structure.

A dynamic clamp for the injection of synthetic conductances into biological neurons 1059

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A neuron has electrical properties that can be measured, and also altered by applying electric charge. The standard approach to varying the state of the membrane is to inject a constant voltage or current step into the inside of the neuron, however this does not reproduce a natural process. The natural flow of ions through the membrane is more complex than a constant current or voltage flow because currents are not carried by electrons, but by various ions, and the channels conducting them exhibit voltage dependency.

A dynamic clamp computes a voltage and time dependant current flow that behaves like a naturally occurring ion flow. The dynamic clamp is connected directly to the inside of a neuron where a voltage is measured and used to calculate the appropriate current injection.

We present the design and implementation of such a dynamic clamp device for biological neurons. The dynamic clamp technique can be used in neurophysiology experiments to add, or subtract, virtual ion channels in living neurons. The device calculates a voltage and time dependent current flow in a closed loop, which emulates the naturally occurring ion channels of a neuron.

The Hodgkin-Huxley ion channel equations were modelled in the Simulink graphical simulation environment. The model is flexible and can be extended without program-

ming knowledge. A graphical user interface was created to facilitate its use in experiments. The level of abstraction achieved using the Simulink environment to describe dynamic clamp creates a portable model that can be run on any other hardware platforms supported by Simulink.

We used a variety of hardware platforms. A Texas Instruments evaluation module using a TMS320C6701 digital signal processor (DSP) was used in the Matlab/Simulink environment. Because the analog to digital and digital to analog converters (ADC & DAC) devices are embedded in the onboard CODEC for this device and use frame-base data this imposes an upper limit on data throughput. This limitation can be removed easily using an appropriate analog daughter card. Alternatively one can use a variety of off-the-shelf data acquisition boards from a variety of manufacturers with the Real-Time Windows Target from the MathWorks.

The dynamic clamp device was tested in open and closed loop configurations on a simulated neuron and found to function correctly. The device was then used in experiments with living rat cortical neurons. The 'whole-cell patch clamp' technique was used to gain access to the inside of the neuron, where emulated sodium and potassium ion channels were injected with the dynamic clamp. This successfully produced artificial action potentials in a living neuron. Several avenues were explored for the further expansion of the device. The most promising is extending the device to include synaptic channels, so that artificial connections can be created between otherwise isolated neurons.

1060 LabTools: An integrated web-based framework for the publication of neuro-scientific data

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The biological sciences in general and the neuroscientific communities in particular are confronted with an increasing need to organize and selectively publish vast amounts of data. Here we present a software tool that we develop to support the sharing of data from research laboratories through the web.

The software runs on all platforms where Python (a powerful scripting language) is available, i.e., Windows, Mac, Linux, and any Unix variant. It enables fine grained control about who is allowed to access and manipulate standard information (who did what when and where) as well as experimentally gained original data using web techology. It does so by providing a so called *workflow tool* that allows site managers to define states (like 'private', 'group visible', 'published') associated to individual object types (like 'data sets'). It is then possible to define who is allowed to view or edit an object depending on this state (e.g., 'group visible' may be set to allow other members of the laboratory to view the data). Furthermore, site managers can define transitions between states including permission settings to control who is able to invoke which transitions. That way the individual researcher (or head of the laboratory) can control in detail who is allowed to access which particular object and these rights can easily be changed as needed.

This solves one of the most prominent current problems in data sharing via the web in that it enables individual laboratories to make their data accessible through the web in principle but without loosing detailed control about who is able to view or download which data. If desired, the software can be configured to support the submission of descriptive meta data about indivdual content items to the neuroinfomatics portal at http://www.neuroinf.de.

Technically, LabTools are a realization of a customized framework on top of Zope (http://www.zope.org), a general object publishing environment, and its content management framework (CMF; http://cmf.zope.org). LabTools can be obtained free of charge and they are distributed under an *open source license* (as are Zope and CMF). The most up-to-date information about this project can be obtained from http://www.neuroinf.de/Members/ritz/LabTools.

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The internet portal for the neurosciences at http://www.neuroinf.ne 1061

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Following the open source philosophy, more and more neuroscientists are willing to share their primary data as well as custom software with the scientific community. To facilitate this interaction, we are establishing a web portal that links publicly available resources (such as experimental data, data analysis tools, computer models) and contains extensive annotation.

Special emphasis is given to high quality standards of the linked resources and to integrate both users and providers in this process.

Building on open source software tools, we therefore implemented a web-based content management system including a reviewing process to enable neuroscientists to contribute to the underlying metadata database. We also developed a neuroscientific classification system. Currently we are incorporating tools to benefit from standardized usage of metadata and from the evolving field of web services. This project is an integral part of the international efforts coordinated by the European Union and the OECD to foster the developing field of computational neuroscience and neuroinformatics.

Our long-term goal is to contribute to a global internet portal for the entire field of neuroscience with a particular emphasis on facilitating the exchange of data and software but also providing various other kinds of information, such as *who is doing what and where* or services like news, bulletin boards and threaded discussions. The current state of the project can be checked at http://www.neuroinf.de.

We do not plan, however, to serve as a primary data repository where people can upload and publish raw data sets for instance (but see our accompanying contribution on LabTools). All we are interested in with the portal is the collection and dissemination of so-called metadata, meaning structured data about data. People can use the portal to adver-

tize their resources which are already available somewhere else on the internet. The potential benefit of doing so is to become more visible to the community.

The poster presents an overview over the development of the portal and will also serve as a meeting point for people interested in discussing and possibly joining the various project activities.

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1062 Automatic Extraction and Visualization of Functional Information from Autoradiographic Brain Image Stacks

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The authors present a new method for the automatic segmentation as well as the automatic extraction and 3D visualization of functional information from autoradiographic image stacks, such as 2-fluoro-deoxyglucose (2-FDG) images. The difficulty in the evaluation of these so-called "2 ½ D" datasets is that they do not inherently represent a continuous 3D data volume (as MRI or CT), but consist of a stack of images from single tissue slices. From the image processing point of view, this corresponds to data consisting of a series of consecutive, distorted gray value images, while the exact position and direction of the original slices varies among different datasets. This complicates the comparison of different experiments, if the evaluation is performed by considering single slices only.

We deal with that problem by introducing an automatic method for segmentation and 3D-surface reconstruction of the brain areas, followed by the automatic extraction and visualization of functional information (originally encoded in the image gray values) as a texture mapped to the cortical surface. The proposed method leads to an abstraction from the single-slice nature of the individual datasets, allowing the researcher to look at the 3D anatomy instead of slice image stacks and is furthermore capable of visualizing functional information from autoradiographic brain image stacks in form of activity surface maps.

A widely used approach for the functional analysis of 2-FDG-images is to look at the gray value profiles along a path chosen by the researcher, but the resulting activity profiles are governed by noise. Instead, we introduce the concept of "Map Space", which provides a parametric representation of the cortex in a rectangular (normalized) coordinate system by the use of an optimal mapping function, whose parameters automatically adapt to the local shape and curvature of the cortex. The functional information is evaluated within this parametric representation in Map Space by averaging the gray values along the coordinate axis corresponding to the cortical depth. This leads to a reduction of noise and allows the visualization of cortical activation as texture on the surface of the reconstructed cortex. Because this 3D model is almost independent of the position

and direction of the original slices, not only the localization of activation sites is more accurate, but also the comparison of different individuals is made possible.

The general method of segmentation and generation of texture maps containing activation patterns is applicable to different kinds of autoradiographic datasets. In this work, it will be demonstrated using 2-FDG-image stacks from rat brains under acoustical stimulation.

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Eye Movements in Comatose Patients - Galvanic Evoked Vestibulo-Ocular Monitoring - 1063

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We expected a dependence of eye movements from an induced vestibular stimulus in comatose patients. This function (the relation between eye movement and vestibular stimulus) should be interindividually constant in healthy persons. A pathology of the vestibular integrating structures (brainstem, but as well cerebellum and cortex) should influence this function pathognomically and reproducibly .

To prove this hypothesis in this project a galvanic current is applied to comatose patients to induce a vestibular stimulus (GaLa). The resultant eye movements are digitally recorded and processed (VidOc).

The vestibulo-ocular reflex is one of the robust reflex arcs in man: The vestibular nuclei in brainstem are innervated by the vestibular nerve. The vestibular nuclei send information to the ocular muscle nuclei in brainstem. Over this pathway reflective eye movements can be elicited.

For GaLa electrodes are attached over the right and left mastoid. The electrodes are fixed on the skin surface of the patient. Further two electrodes are fixed interscapular on the back of the patient. The electrodes of mastoid and shoulder of one side compose a pair for stimulation. By this technique both labyrinth can be polarized independently. A custom-built battery driven stimulator generates sine wave or trapezoid currents. We use currents with an amplitude of up to 8mA and a frequency of 0,5Hz. This current is employed to provide independent, binaural polarization to the left and right labyrinth. It is possible to tune in a phase-shift right-to-left by 90, 180, 270 and 360deg.

Eye movements of the left and right side are recorded independently. Binocular recording is performed using a head fixed infra-red digicam eye tracker. We use a sample rate of 50Hz for each eye. The system generates a data file for each recording.

The VidOc data files are analyzed by a specialized software (iris tracker, Chronos Vision, Germany). Using contour analysis the pupil as well as a segment of the iris are defined. An analysis of vertical-, horizontal- and torsional movement is performed off line. This 3D movement data can be processed further by mathematical computer software.

We investigated repeatedly five comatose patients with Glasgow Coma Scale of 3, who were intubated and ventilated (subarachnoid haemorrhage (n=3), traumatic injury (n=2)). The observed ocular movements varied between the patients. In four cases an ocular movement as response to GaLa was observed, while one patient showed no eye movements, neither spontaneously nor as galvanic response. This patient was diagnosed brain dead 20 hours after the vestibulo-ocular monitoring. Another patient who showed spontaneous oscillation and ocular movements synchronous to galvanic stimulation left the intensive care for rehabilitation.

These data indicate that it is possible to assess brain function of comatose patients by galvanic vestibulo-ocular monitoring.

1064 The *monist-project* -- Educational Simulations For Brains

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To understand the functioning of brains we must bridge many levels of analysis from molecules, cells and synapses to perception and behavior. Although experimental analysis is a precondition for understanding information processing by nervous systems, it is in no way sufficient. Rather, computer simulations help to tackle problems resulting from dynamics and complexity of nervous systems. It has become common scientific practice in the neural and cognitive sciences to investigate brain function by both experiments as well as model simulations.

In the higher education of the neural and cognitive sciences, however, simulations are still only applied sporadically – even though educational materials (e.g. internet applets for well-known models such as Hodgkin-Huxley, Reichardt-detector, LTP etc.) and simulation tools (e.g. MatLab™) are readily available. Application suffers from various uncertainties of how to use simulations in higher education: *Do simulations effectively complement the well established methods of "chalk and talk", laboratory practicals or paper discussions? How long will it take to prepare application? Exactly which cases have a reasonable cost-gain ratio? Should I make demonstrations or let the students make it by their own in a practical course? How can I assess success or failure of use...* In sum, 'recipes' for applying simulations in education are widely missing.

The *monist-project* has committed itself to the task of developing such recipes for simulations in higher education. The basis of the *monist-project* is a software package (*monist-console*) designed for providing compact, ready to use educational simulations. Simulations become educational as they are complemented by topical texts, instructional design, concrete tasks and an editor for individual notes and solutions. Thus, the *monist-console* supports self-learning but particularly aids tutors to integrate simulations in their courses. Online file-management and communication tools (via *monist-server*) help to organize personalized courses.

The authoring mode provides means for designing or modifying educational material. Existing simulations (applets, programs etc.) can be embedded. An integrated simulation construction kit is under development. Authored educational simulations can be sub-

mitted to a central server for publication. An update routine can offer newly published simulations on the server for download to the local *monist-console*.

Beyond software and content development, the *monist-project* works on several practical fields for fostering the concrete application of simulations in higher education, e.g. evaluation for best practice scenarios, studies on learning and memory and organizational networking. The demonstration of the *monist-project* offers applicable tools and content (work in progress). It aims at teachers wishing to apply and evaluate the *monist-console*, authors seeking to contribute concepts or integrate their existing simulations and developers ready to participate. The *monist-project* is funded in nine groups at six universities from 2001-2003 by the German Federal Ministry of Education and Research *BMB+F*. One group carries out evaluation (Seel, Freiburg), the others correspond to topics, as follows (alphabetically, contributing groups in brackets):

Biophysics (Aertsen, Freiburg) Cognitive Neuroscience (Mallot, Tübingen) Learning and Behavior (Menzel, Berlin) Motor Systems (Cruse, Bielefeld) Neural Networks, Applied (Gross, Ilmenau) Neural Networks, Theory (Ritter, Bielefeld) Psychology (Dörner, Bamberg) Sensory Systems (Egelhaaf, Bielefeld)

Further information: http://www.monist.de

3D-Reconstruction of insect flight trajectories from 2D image sequences 1065

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For the detailed analysis of the flight behaviour of insects, the flight trajectories have to be reconstructed from experimental data.One frequently used source of data are digitised video sequences.To reconstruct the trajectory in 3D, at least two synchronously recorded different views of the flight must be analysed. To resolve rapid flight manoeuvres, high frame rates are required, leading to large amounts of raw image data. Manual evaluation of this data is virtually impossible for the large numbers of experiments needed for statistical analysis.

We present the free software package FlyTrace, a package for computer aided reconstruction of 3D trajectories of flying insects from pairs of synchronous image sequences. The only requirement for the camera setup is that the optical axes of the cameras have to be perpendicular to each other.

The image processing component of the package locates the insect in each frame in front of an arbitrary background and calculates 2D-position and -orientation of the animal. If more than one animal is in the view, the tool can automatically detect which animal in the current image is most likely the successor of a given animal in the previous frame. The user interface of the tool is designed to make manual inspection and correction of the automatically generated proposals easy.

A separate tool is used to merge two 2D trajectories generated by the image processing to one 3D trajectory based on the camera positions, which are calculated from simple

calibration images. This processing step calculates the Euclidean coordinates of the animal's position from the pixel coordinates in the two perspective projections.

The software was successfully used in various studies on flying blowflies. These range from laboratory situations such as flights in a flight tunnel or the pursuit flights in the chasing behaviour of male flies to flights under open air conditions. The images were either recorded by standard video cameras or high-speed cameras recording 500 images per second.

The FlyTrace software package may be applied to almost arbitrary moving objects, such as other insects, but also vertebrates such as mice.

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1066 Formation of denervation supersensitivity in rabbits following intraocular and intravitreal injection of botulinum toxin

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Denervation supersensitivity is the enhanced contracted response of the striated or smooth muscle tissue following chronic neurotransmitter depletion or destruction of neurons within an organ. Development of denervation supersentivity to acetylcholine occurs after disruption of innervating motor neurons in skeletal muscle.

Botulinum neurotoxin (BoNT) is a member of a group protein toxin binding to the cell surface that becomes internalized in vesicular compartments. It translocates its catalytic subunit into the cytosol at the nerve terminal, and causes a sustained block of acetylcholine release with ensuing paralysis. Cholinesterases are a group of enzymes positioned at the nerve terminal ends and synapses. While AChE (E.C. 3.1.1.7) is the main enzyme responsible for the acetylcholine hydrolysis, the function of BChE (E.C. 3.1.1.8) is not clear.

The intramuscular injection of botulinum toxin-A is used as a therapeutic agent against many neurological disorders associated with the involuntary spasmodic contractions of muscles such as strabismus, facial dystonias etc. In this study, BoNT/A was injected to New Zealand rabbits' eyes through intravitrael and intraocular means as a possible therapeutic agent against strabismus. The effects of these injections on the formation of denervation supersensitivity were investigated through the cholinesterase enzymes, and also checked with the physical evaluations by direct ophtalmoscopy.

The findings show that there was no significant difference between the left and right eyes of rabbits. The route of botulinum delivery was found to be effective on the duration and formation of denervation supersensitivity. As compared with the t-test, intravitrael botulinum injections were significantly more effective than intraocular injections ($p>0.05$). The formation and duration of denervation sensitivity was also evaluated through pupillar measurements with different commercial ChE inhibitors leading to a

period of 4 months. The results obtained with eserine, pilocarpine and arecholine were compared for both routes of botulinum toxin delivery. The intraocular delivery of botulinum toxin displayed conflicting results with eserine and pilocarpine. The finding of myosis response with eserine leads to the fact that only partial denervation is caused through this delivery. The difference in ChE activities unexpectedly arose mostly from BChE not AChE. These findings support the fact that intravitrael injection is much more effective in the formation and duration of denervation supersensitivity and that BChE has yet an undefined role in retinal networks

Qualitative analysis of skilled forelimb use in a modification of the paw-reaching test in rats 1067

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This study aims to show how the paw-reaching test can be used for qualitative in addition to quantitative analysis of skilled forelimb use. So far, the paw-reaching test has been performed with standardised food pellets allowing counting of the pellets remaining in the staircase box on those stairs where pellets were placed beforehand in comparison to those pellets dropped elsewhere in the box. Out of that numbers, the rate of pellets taken, successfully grasped and eaten was measured. However, below the defined number of ten pellets per stair, it is difficult to measure whether a pellet from one stair has been shifted to another. Thus, the relation of pellets taken to pellets eaten cannot be exactly analysed. We now refined the counting method with differently coloured pellets for each stair. This allows not only to achieve more exact counting, but also to perform a detailed analysis of the dropping pattern of food pellets, comparing each stair not only for remaining and eaten pellets, but also for the number and colour of misplaced pellets. Comparing healthy rats with animals bearing a unilateral, complete 6-hydroxydopamine lesion of the right nigrostriatal pathway, we assessed the impact of this modification in two testing contexts (free and forced choice). As the level of grasping difficulty increased, the lesioned animals showed significantly more mistakes, and a lowered grasping activity compared with healthy controls. The diversity between the old and the new counting method was most pronounced on the lower stairs, revealing a significant improvement in the differentiation of skilled forelimb movements in normal and lesioned animals. This might also became highly useful when studying the restorative capacity of intracerebral neural grafts.

MRI-compatible, headmounted platform design for use in small laboratory primates 1068

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A concept is presented for the construction of MRI-compatible, headmounted platforms describing moulding, implantation technique and headstage design. It gives instructions

for production and assembly, including descriptions of necessary workbenches. Although the concept was originally developed for use in bio-telemetry in the squirrel monkey (*Saimiri sciureus*), the general principles of making and implantation allows its use in other experimental animal species too. Advantages are low weight and costs, individually adjustable design and standardized production. The construction is entirely composed of synthetic material. The required materials are sterilisable and biocompatible. The main principles of the method are:

1. the usage of a skin excision template with the same diameter and size of the cylindric platform mount which allows exact wound closure after implantation;

2. a simple technique for single step in-place moulding and fixation of a cylindric platform mount on the skull of an experimental animal. Applying the method, cuts down time for surgery, implantation and anaesthesia in our laboratory by half. The moulding procedure applied in this technique is superior to casting. Moulding creates a perfectly smooth surface of the dental acrylic rim in contact with the skin, leading to a recessus-free, small skin-implant interface and hence minimizing the risk of wound infections as a possible complication of longterm (over one year) implanted platforms;

3. the moulding and fixation of a mount in a first step surgical procedure, which allows the fixation of the actual platform in a second step without any additional surgery;

4. a simple and low cost 'do it yourself'-technique for platform moulding easy to perform in any laboratory. The shape and size of the platform to be mounted can easily be adjusted to the particular requirements by moulding or later reshaping.

1069 Construction and performance of a custom-built two-photon laser scanning system

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Two-photon microscopy enables non-invasive studies of single cells inside living tissue at submicron resolution, and the restriction of two-photon excitation to a tiny focal volume gives to visualization, measurement, and photopharmacological studies an intrinsic, three-dimensional resolution. Therefore, there is a considerable interest in optimizing the performance of two-photon laser scanning microscopes (TPLSMs). Commercial TPLSMs are often offered as an extension to a conventional laser scanning microscope. These systems are, however, relatively cost intensive and their construction is fixed, which considerably complicates upgrading. In addition, being designed as multi-purpose instruments, their optical paths may not be optimal for two-photon excitation. They generally contain a large number of optical components, all of which may have a negative effect on laser power, the spatial profile of the laser beam and especially the duration of single laser pulses, i.e. those parameters critical for spatial resolution. We therefore decided against the purchase of a commercial system and started ab initio, setting up a TPLSM from single components. In this presentation we would like to share our experience with others. Construction details of our custom-built system are given and its performance is demonstrated. The system was designed for simultaneous optical

and electrophysiological recordings, and the illumination path was optimized in view of power-delivery and laser pulse-broadening by introducing only a minimal number of optical components. For this purpose, a solid-state pumped, mode-locked Ti:sapphire laser was directly coupled into a modified upright microscope. The scan unit was built around commercial scanners and a Zeiss scan lens. For maximum detection efficiency fluorescence was detected in non-descanned mode by a photomultiplier tube. Many mechanical parts, electronic circuits and the software for system control and image acquisition were developed in our lab and can be readily modified according to special needs of experiments. All components are easily accessible and can be upgraded according to optical requirements. Due to the optimized illumination path 100 fs laser pulses are broadened by a factor of two only, yielding a final pulse length at the sample of 200-220 fs. Two-photon excitation was verified over the entire wavelength tuning range (750-975 nm) using fluorescence particles and fixed cell preparations. From the fluorescence-intensity profiles of subresolution beads (100 nm) we determined the point-spread function and a lateral and an axial resolution of 0.48 and 1.44 µm, respectively. Thus performance is comparable to available commercial systems, but our TPLSM is superior in many aspects of cost, flexibility and versatility. Currently the two-photon system is being used to investigate the organization of mitochondria and their subtle interactions with other cell organelles in brainstem neurons cultured from respiratory center (see our second presentation).

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Sub-microscopic mitochondria motility in mitral cell dendrites studied by single particle tracking 1070

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Mitochondria motility is critical for neuronal functioning. They generate ATP and take part in the regulation of intracellular calcium levels. In this study we have examined mitochondria motility in dendrites of cultured neurons of *Xenopus laevis* tadpoles using single particle tracking (SPT). SPT has been used largely in biophysical research to observe the diffusion of small lipids and proteins in cell membranes. The common SPT algorithms can be used if the object being tracked has a constant size and, in case of a Gaussian-fit algorithm, a diameter much smaller than the wavelength of the focused laser beam. However, the standard algorithms fail if the particle changes shape, size or orientation during tracking. We developed an algorithm that takes into account size-fluctuations of the object being tracked and studied sub-microscopic displacements of dye-labeled mitochondria in dendrites. Our data reveal a correlation between movement and sub-microscopic size-fluctuations of mitochondria.

1071 Ratiometric confocal calcium imaging in developing insect neurons using Fura Red

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In the past, ratiometric confocal calcium imaging was performed by either using dyes like Fura-2 that require UV illumination, or by using the combination of two dyes like Fura Red and Fluo-3. Both methods, however, have drawbacks like the high costs of UV lasers, significant photo damage of the tissue by UV light, and the changing ratio of the two combined dyes throughout the experiment, respectively. Here, we describe a new method to ratiometrically measure calcium with confocal laser scanning microscopy by using a single dye, Fura Red, and visible light.

Neurons *in situ* of the developing olfactory lobe of the sphinx moth *Manduca sexta* were bulk loaded with 3 μm Fura Red-AM for 30 min. For calibration, the cells were permeabilized by a mixture of ionomycin (50 μm) and 4-bromo A23187 (20 μm), and the fluorescence ratio was determined for Ca^{2+}-free and Ca^{2+}-saturated conditions. Fura Red was alternatingly excited at 457 nm, which is close to the isosbestic point of Fura Red, and 488 nm, where Fura Red shows a large decrease in fluorescence upon binding of Ca^{2+}. The fluorescence was measured through a 585-nm long-pass filter. From the fluorescence ratio F457/F488, the Ca^{2+} concentration in the cells was calculated.

The results show that application of 100 μm Carbachol for 5 minutes induced Ca^{2+} oscillations in the neurons in stages 4 to 9 of metamorphic development, while later in development the neurons responded only with a single Ca^{2+} transient. From stage 4 to stage 9, the frequency of the Ca^{2+} oscillations increased from 0.6 to 1.4 events/min. The Ca^{2+} oscillations were blocked by d-tubocurarine and cadmium, indicating that they are due to nicotinergic excitation leading to the opening of voltage-operated Ca^{2+} channels.

1072 Single-cell electroporation of HEK293 cells and auditory brainstem slices

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The auditory brainstem plays a pivotal role in the processing of auditory information and represents an attractive model system to study synaptic transmission, as well as the development of neuronal circuits and neuronal plasticity. To investigate the neuronal elements in more detail, we have previously established an organotypic slice culture of the pontine auditory brainstem, i.e. the superior olivary complex (Lohmann et al., 1998). In combination with an efficient technique for gene transfer, this slice culture will be a useful tool to identify molecular mechanisms involved in the above aspects. Here, we report on the establishment of the single-cell electroporation (SCE) technique, which allows targeted gene transfer into individual cells in intact tissues using a modified patch-clamp methodology (Haas et al., 2001). Under optical control, the tip of a micro-

pipette filled with DNA is brought into close vicinity of the cell body of a single cell. Upon electrical stimulation, the DNA is then delivered into this cell.

In order to set up SCE, we first electroporated cultured HEK293 (human embryonal kidney) cells with the plasmid of the enhanced green fluorescent protein (eGFP, Clontech) as a reporter gene. Various parameters, such as voltage amplitude (4-10 V), pulse width (1-2 ms), pulse frequency (100-200 Hz), pulse duration (1-3 s), or pipette resistance (8-20 MOhm) were tested for best transfection efficiency. Many combinations resulted in a similarly high transfection rate of more or equal than 50% (checked after about 48 hrs), as long as the average dissipated power (I^2 x R x duty cycle) was about 0.6 µW. These results confirm previously published data (Rae and Levis, 2001). Next, we examined the influence of the plasmid size on transfection efficiency. No significant difference was observed when the plasmid size was varied between 4.7 and 8 kbp. To establish SCE in brainstem slices of mice and rats, we performed electroporation of single neurons in acute slices using the fluorescent dye tetramethylrhodamine dextran (3,000 Da, anionic; Molecular Probes). As no gene expression is required, the electroporation efficiency could be immediately monitored. Inspection of the slices shortly after electroporation revealed an efficiency of more or equal than 90%. We are currently extending our study to organotypic brainstem slice cultures. These experiments will be performed in order to overexpress or silence a given gene. In summary, our results indicate that SCE represents a promising technique to genetically manipulate a subpopulation of selected cells in order to analyze their gene function.

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Computer controlled multiple odour sources for defined antennal stimulation 1073

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Electroantennogram (EAG) setups are in use for a large number of experiments. Recently, an EAG system has been constructed to measure airborne pheromone density in mating disruption experiments [1]. For further insight into receptor mechanisms, it seems worthwhile to study the interaction of odours using controlled superposition of odour stimuli. For such experiments it is essential to use stable and reproducible odour sources. In standard procedures filter papers are used [2], but these sources lack reproducibility [3]. A syringe containing varying ratios of oil and odour material is an odour source with the required stability [4]. If such syringes are to be applied in a superposition experiment, they should be positioned all at the same distance from the antenna (i.e. in one plane) to achieve the same stimulating effect. Thus, only 3 syringes can be permanently connected to a central air channel leading to the antenna. This layout was used in the pheromone measuring system [5]. Since at least 2 syringes are needed for the calibration of the antenna, the old system can measure the superposition of only 2

odours at one time. For more comprehensive measurements of odour interactions it is desirable to apply many different odours in a short sequence, since the antenna's sensitivity and threshold vary and intervals between calibrations must be kept small. Since 10-15 syringes are desirable, the existing construction had to be changed dramatically. In the new system one syringe is replaced by a barrel carrying 12 syringes. The barrel is rotated by a motor with position feedback. Thus, each of the 12 syringes can be selected and pushed into the injection hole. Air puffs from the syringes in use are generated by moving the piston by a step motor, which gets defined impulses from a microcontroller. The step motor's feed rate controls the concentration of the odour at the antenna. In order to get interaction between all 3 odour sources in use, the step motor control system permits to activate each motor independently with its own feed rate, with total or partial activity overlap. All functions of the system are remotely controlled by a PC which also records the EAG trace.

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1074 Volume Warping of Segmented Brain Data Sets in Autoradiographic Imaging

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An accurate comparison of inter-individual volumetric image data sets of brains requires geometric transformation techniques (warping) to reduce shape variations of the individual brains. In this study we use so called model-based warping methods, based on corresponding surfaces of manual segmented brain structures to perform the transformation. The transformation function used here is based on distance-weighted warping methods. Such surface-based warping methods are published for human brain data sets (Thompson & Toga, (1996) IEEE Transactions on Medical Imaging. 15(4): 1-16). But in the case of warping with surfaces of certain inner brain structures a manual segmentation is necessary. It is time consuming but the gives the advantage to incorporate expert knowledge especially for a better labeling of low contrasted brain regions. The approach was tested on reconstructed 3D autoradiographs of brain data sets of 6 individual gerbils.

The autoradiographs were obtained after interperitoneal injecting of a non-metabolizable radioactive sugar (2-FDG) into gerbils. 2-FDG mainly visualizes excitatory brain activity. Afterwards, the removed brains were cut and processed with standard autoradiographical methods (Scheich et al. (1993) European Journal of Neuroscience 5: 898-914) and digitized. By means of a three dimensional reconstruction process the slices were aligned to recreate the original brain geometry. Next a certain number of brain structures

(Septum, Striatum, Hippocampus, Thalamus and outer contour) are manual segmented slice by slice and surface models of all structures for each brain are generated by triangulation. Afterwards, the reference system is defined as the average of all individual surface models. For generating this average surface model the spatial locations of corresponding nodes (nearest neighbour) of the triangles of the individual surface models are averaged for each structure separately. The spatial difference between each surface triangle of the individual models and the corresponding (nearest) one of the reference system defines a displacement vector. Afterwards the volume warping uses the obtained displacement vector field to transform the given 3D data set. Next, the similarity between the data sets was measured by a 3D linear cross correlation coefficient (densitometric similarity) and volume overlap index (shape similarity).

Before warping the mean cross correlation coefficient between the individual data sets was 0.6955 and after warping 0.7336 (increase of 5.48%). The mean volume overlap index was before warping 0.9119 and after warping 0.9438 (increase of 3.50%).

The described approach combines a manual segmentation, surface rendering and volume warping to reduce interindividual shape variations of autoradiographed gerbil brain data sets. The similarity after warping regarding the used similarity functions is better than previously published approaches using grayvalue-based edge detection methods instead of manual segmentation (Pielot et al. (2002) Bildverarbeitung für die Medizin 2002 318-321).

However, application of these fully automatic procedures is much faster than manual segmentation, so that they are mainly suitable for fast analysis of large groups of data sets. For most accurate analysis of smaller numbers of data sets the presented procedure may prove to be a useful tool to make biological structures accessible to a quantitative densitometric evaluation.

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FLIM at Minimal-Invasive Conditions: Ultra-Low Excitation Levels and Ultra-Sensitive Imaging Detectors 1075

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Standard investigations, analysing the dynamics within living cells, are most frequently based on time-lapse, video or confocal laser scanning microscopy in combination with vital fluorescent markers and complemented by intensity-based FRET or FCS analysis. Unfortunately, the application of scanning systems in fluorescence microscopy (often using fs lasers) and the strive to collect as many photons as possible from a single molecule (e.g., during passage through a small excitation volume, FCS), necessarily results in high average and peak-powers, which lead to irreversible photobleaching of dyes, induction of photodynamic reactions, and significant photodamage of living cells. Therefore, these standard visualisation techniques (i) do not allow the direct observation of single molecular interactions over a longer period of time, (ii) induce photodynamic

reactions of the molecular probe (e.g. mimicking FRET by inducing GFP-conformational changes) and (iii) distort the living state.

Consequently, the development of minimal-invasive observation systems that yield information about protein-target structure interactions, without disturbing the intracellular metabolism, is an outstanding goal in modern cell microscopy.

Novel ultra-sensitive, non-scanning imaging detectors, based on time- and space-correlated single photon counting (TSCSPC) allow ultra-low excitation levels and FLIM observation of protein-protein, DNA-protein, DNA-DNA, and membrane-protein interactions in living cells at minimal-invasive conditions that preserve the living state.

Here, we describe a novel laser-based multi-parameter fluorescence microscope, which consists of a novel ultra-sensitive, non-scanning imaging (250 x 250) dual-detector unit that works at ultra-low excitation intensities (average power: 10-100 mW/cm2 in living cells, being 1.000-10.000 less than in standard systems) to provide:

- Simultaneous dual-colour, loss-free FLIM and FRET observation
- Simultaneous dual-polarisation, loss-free picosecond anisotropy imaging for simultaneous determination of rotational constants and homo-FRET
- FRET-verification by applying picosecond-resolved emission spectroscopy.

The multi-parameter fluorescence microscope provides EPI- and TIRF-illumination and is currently used to study XFP-fused (XFP = CFP, YFP, GFP, DsRed, HcRed) proteins that are involved in signal transduction cascades within living neuronal cells. In combination with a laser-based micro-manipulation scheme, the system provides a unique tool to study protein-target interactions down to SM level under minimal-invasive conditions.

1076 Fluorescence Lifetime Imaging Microspectroscopy of xFP-fused Proteins in Hippocampal Cell Cultures using Ultra-Low Excitation Levels and Ultra-Sensitive Imaging Detectors

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Understanding functional changes within living neurons (e.g. during synapse formation or signal transduction cascades) requires a precise investigation of the molecular interactions between macromolecules within their natural environment. Previous investigations for studying the dynamics within living cells were based on the use of video or confocal laser scanning microscopy in combination with vital fluorescent markers. Unfortunately, most of these visualisation techniques (fluorescence/CLSM/2-Photon, etc) use high illumination intensities that cause considerable photo-dynamic reactions

within living cells and, therefore, do not allow a non-distorted continuous observation of dynamic processes for a longer period.

With the introduction of novel ultra-sensitive delay-line (DL) and non-scanning imaging detectors (QA = quadrant anode), based on time- and space-correlated single photon counting (TSCSPC), recently, it has become feasible to overcome the limitations of standard fluorescence microscopy and to follow the dynamics of biological processes in living cells without inducing considerable phototoxicity, i.e. at minimal-invasive conditions that preserve the living state.

Here, we demonstrate for the first time the possibility to use DL and QA detectors to monitor and analyse the spectral and temporal characteristics of xFP-fused proteins within living neuronal cells at ultra-low excitation levels (average power < 100 mW/cm2). As biological targets, we investigate xFP-fusion constructs of small and large neuron-specific proteins (Bassoon: ca. 500 kDa, Caldendrin 33/36 kDa, Jacob: ca. 60 kDa) and their putative binding partners. Previous studies have shown that these proteins play a pivotal role, either as scaffolding constituents within the presynaptic boutons of developing synaptic contacts (Bassoon) or as Ca-sensitive messengers (Caldendrin and its binding partner Jacob) that might be involved in a signalling pathway from the post-synaptic contact to the nucleus. Using various xFP-fusion constructs of these proteins we have analysed their picosecond spectra in living neuronal cells and compared them with the spectra of the corresponding xFPs. Moerover, we have analysed the autofluorescence contribution and xFP artefacts that frequently produce fast FRET signals. For instance, spectroscopic analysis of Caldendrin/Jacob constructs (GFP/DsRed) has revealed that very often autofluorescence or immature dye-components (DsRed1/DsRed2) mimicks FRET. By acquiring time-resolved spectra from selected areas within the same cell, autofluorescence contributions can be recognised and “subtracted” from the FRET-signal. In addition, it is possible to discriminate between fast fluorescence dynamics emanating from immature xFP and specific FRET signals.

Using picosecond spectroscopy and picosecond lifetime imaging, we have further analysed fluorescence kinetics of xFP-fusion constructs of the synaptic cytomatrix protein Bassoon and its interactions with putative binding partners within living neurons. Preliminary imaging data taken with the QA-detector has revealed individual blinking of nm-sized GFP-Bassoon vesicles, indicating that only few GFP-Bassoon molecules are attached to single vesicles.

In conclusion, our preliminary studies with DL- and QA-detectors have shown that fluorescence lifetime imaging microspectroscopy is an excellent tool to analyse FRET between GFP-fusion proteins and putative interaction partners in living hippocampal neurons at minimal-invasive conditions.

1077 Reduction of High Dimensional Brain Signals by Radial Basis Functions for Extracting Differences in the Small-sample Case

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Introduction. The identification of signal components by which two sets of measurements can be segregated is a common task in electrophysiology. A major problem in this field is that the number of stimulus-response pairs, available from an experiment, is often low whereas the dimensionality of the observation space is high. An appropriate data analysis technique has therefore to fulfil difficult constraints. It has to be applicable in high dimensional space and should, at the same time, be effective to small data samples. Further, it has to be robust against noise and outliers, easy to implement, and it should cost low computational effort.

Methods. As a promising choice to achieve these constraints we propose to use radial basis functions, transforming the recorded high dimensional raw data to a low dimension. In doing so a continuous hypersurface will be constructed from a randomly selected subsample of the data sets. For a description of the method and possible applications to local field potential recordings from the visual cortex of awake monkeys see [1]. In the present work we investigate the projection properties and the utilisation of radial basis functions to other electrophysiological signal types. Empirical examinations on artificial data sets with predetermined characteristics are used to compare the performance of this method with other dimension reduction techniques. The information reduction as a result of the dimension reduction will be quantified by several distance measures including mutual information. Emphasis is placed on the dependence on the signal-to-noise ratio and the influence of irrelevant signal components to the segregation performance.

Results. Beyond the reported application to local field potential recordings from the striate cortex of awake monkeys we show that this method can also be used to analyse other signal types, for example multi-unit spike activity (MUA, resembling an amplitude signal of local spike density). As an example we extract differences in multi-channel neuronal signals recorded in monkey striate cortex corresponding to different visual perceptual states (for a description of the experimental methods see [2]). Usage to diverse types of neural signals will also be demonstrated by means of data sets produced with neuronal model networks, consisting of coupled integrate-and-fire neurons [3].

Conclusions. Our results indicate that this approach is applicable to numerous multichannel signal types. Furthermore it can be combined with other preprocessing techniques, e.g. to achieve a better signal-to-noise ratio.

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Towards Cell Selective Staining with Voltage Sensitive Dyes Using Enzyme Activation 1078

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Voltage Sensitive Hemicyanine Dyes are well established fluorescent probes for measuring fast changes of the voltage in nerve membranes. Their application in brain is limited due to non-selective staining of the tissue. Here we describe a model experiment that may eventually lead to a selective staining of individual nerve cells by enzymatic activation of a water soluble dye precursor.

Three steps are considered:

(i) An amphiphilic hemicyanine dye with and without phosphoric acid ester headgroup is synthesized. The partition coefficients between lipid membrane and water are determined for both species by lipid titration relying on the enhanced fluorescence quantum yield of membrane bound dyes. The lipophilicity of the phosphorylated dye is lower by a factor of 15.

(ii) An alkaline phosphatase converts the phosphorylated dye quantitatively as shown by HPLC. In a suspension of small lipid vesicles, the enzyme induces an increase of fluorescence that reflects a Michaelis-Menten kinetics with subsequent binding of the dephosphorylated dye to the lipid. The kinetic parameters K_M and k_{cat} determined by fluorometry are confirmed by microcalorimetric measurements in the absence of lipid.

(iii) Individual giant lipid vesicles (diameter 20_{mikrom}) attached to a solid substrate serve as cell models. They are incubated with the phosporylated hemicyanine. Addition of phosphatase induces efficient staining as indicated by an increase of membrane fluorescence by a factor of about 20 in microscopic images. The model experiment forms the physicochemical basis for development of enzyme induced staining with genetically targeted cells.

Comparison between different stimulus identification techniques 1079

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Understanding the spatio-temporal receptive field structure of nerve cells typically involves the application of random stimulus waveforms and looking at which stimulus aspects excite the neuron most effectively. These aspects can be obtained from the stimulus ensemble preceding a spike by reducing the dimensionality of this ensemble in different ways. Here, we quantitatively compare the results of the various techniques described in the literature.

The methods that we choose to compare start by separating out those segments of the stimulus that precede a spike. Doing this for all the spikes in a train results in a stimulus ensemble. The spike-triggered average is simply the average of this ensemble and is identical to the cross-correlation between stimulus and spike train. Principal component analysis (PCA) starts with calculating the covariance matrix from the stimulus ensemble and then determines the eigenvectors of this matrix (Simonds, 1963). As a slight variation of the method, the covariance matrix may also be corrected for random stimulus episodes before calculating the eigenvectors (Brenner et al. 2000). The resulting principal components denote those stimulus features that, depending on their amplitude, are most effective in driving the neuron. However, the requirement of orthogonality in PCA may bias the analysis. As an alternative, independent component analysis (ICA, Bell and Sejnowski 1995) relaxes the constraint of orthogonality in favor of statistical independence, but requires prior information about the number of components in question.

Applying the above methods to an experimental data set obtained from the H1 visual neuron of the blowfly (Borst 2003) we compare the results provided by the different methods with the goal to understand how the resulting stimulus waveforms relate to one another, thereby demonstrating the appropriate method for different circumstances. We also discuss the relevance of these methods for understanding the underlying neural code.

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1080 Efficiently maturing and circularly permuted variants of the sapphire mutant of GFP

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The Green Fluorescent Protein (GFP) has been a crucial constituent of genetically encoded signal transduction indicators and of fusions to monitor protein-protein interactions. Mutagenesis of the wildtype gene has yielded a number of improved variants such as EGFP or color variants suitable for fluorescence resonance energy transfer (FRET). However, folding of some of these mutants is still a problem when expressed at 37°C or when targeted to certain organelles.

Sapphire is a mutant of GFP in which the T203I mutation abolishes the second excitation peak at 475 nm that can be found in wildtype GFP. As a result, the mutant protein exhibits a huge Stokes' shift, with an excitation peak at 399 nm and an emission peak at 511 nm. By incorporating a series of folding mutations we produced an improved variant of Sapphire that matures more efficiently and expresses better in E. coli at 37°C. Its

absorbance is pH-stable and the pKa of its fluorescence emission is 4.9, making it very resistant to pH-perturbations inside live cells.

Circular permutations of “Turbo-Sapphire” have acceptable quantum yields and extinction coefficients. As they are supposed to differ in the orientation of their fluorophores in relation to the non-permuted versions they should be ideal tools in optimizing FRET efficiency in new protein fusions as FRET strongly depends on the orientation of the donor and acceptor fluorophores to each other.

“Turbo-Sapphire” and its circular permutations can be used as reporter genes, protein labels and FRET donors in combination with red-fluorescent acceptor proteins such as DsRed, making it possible to completely separate donor and acceptor excitation and emission in FRET experiments.

Spike-sorting with a Bayesian approach implementing a Markov Chain Monte Carlo method. I: Definition of a realistic model for data generation. 1081

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A probabilistic data generation model which includes a description of non-trivial (i.e., non Poissonian) neural discharge statistics as well as a description of spike waveform dynamics (e.g., the spikes amplitude decays upon short inter-spike intervals) is proposed. The discharge of each neuron is described by an homogenous renewal point process, that is, a process fully characterized by its inter-spike interval (ISI) probability density function (PDF): $I(i, \theta_{isi})$, where i is the ISI value and θ_{isi} is the set of parameters required to specify the density. In practice we found that a log-Normal density depending on two parameters gives a reasonably good first approximation to empirical ISI histograms. Following Fee et al (*J Neurophys*, 1996, 76, 3823), we modeled the spike amplitude dependence on the ISI with an exponential relaxation: $A(i, P, \delta, \tau) = P (1 - \delta \exp(- i / \tau))$, where A would be the observed amplitude in the absence of recording noise, i is the ISI, P is the maximal amplitude, δ is the "maximal" amplitude modulation and τ the relaxation time constant. If we assume that the observed data are corrupted by a statistically independent Gaussian noise with a standard deviation of 1, we obtain for the PDF to observe an ISI i and a spike of amplitude a, conditioned on the parameters' values:

$$p(i, a \mid \theta_{isi}, P, \delta, \tau) \propto I(i, \theta_{isi}) \exp(- 1/2 \| a - A(i, P, \delta, \tau) \|^2)$$

We will show how to compute the likelihood function of an actual spike train with N spikes, after the data have been "augmented" by a *latent variable*, $Z = (z_1, ..., z_N)$, corresponding to the membership of each spike (i.e., $z_j = q$ means that spike j has been attributed to neuron q of the model). We will call this latent variable the *configuration* of the spike train. It is then straightforward to obtain the posterior density of the model parameters *and* the configuration conditioned on the data. This posterior density cannot be

evaluated analytically, because too many configurations should be considered (e.g., for a model with K units one has K^N). But we will show that the problem is analogous to the uni-dimensional Potts model used in Statistical Physics. Solutions developed in the latter field can be tailored to the spike-sorting problem with our data generation model. One solution based on the "heat bath algorithm" (as Physicists call it) or the "Gibbs sampler" (as Statisticians call it) will be presented in the companion poster.

1082 Spike-sorting with a Bayesian approach implementing a Markov Chain Monte Carlo method. II: Gibbs sampler based posterior density estimation and consequences for extra-cellular data analysis.

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We propose in a companion poster a more realistic data generation model than the ones commonly used in a spike-sorting context. The key features of this model is that it takes into account both the inter-spike interval probability density of each neuron and the dependence of the amplitude of the spikes generated by a given neuron on the elapsed time since the last spike of the same neuron. The problem with this "sophisticated" model is that it leads to an analytically intractable posterior density for the model parameters *and* the spike train configuration conditioned on the data. Here the spike train configuration is a *latent variable*: $Z = (z_1, ..., z_N)$, where $z_j = q$ means that spike j of the train is attributed to neuron q of the model (we assume that the sample contains N spikes). The analytical intractability is circumvented by estimating $p(\theta , Z | Y)$, where θ stands for the model parameters and Y for the data, with a Monte Carlo method. That is, we cannot compute $p(\theta , Z | Y)$, but we can *sample* from it. This sampling of the posterior density is based on a Markov Chain evolving in the space of model parameters *and* of configurations. This Markov Chain is built such that its unique stationary density is the posterior density $p(\theta , Z | Y)$. The way to build the transition density of the Markov Chain will be illustrated. Once the transition density is obtained, a realization of the chain is simulated with the Monte Carlo method, we are therefore dealing with a Markov Chain Monte Carlo (MCMC) method (Gilks et al, 1996, *Markov Chain Monte Carlo in Practice*, Chapman & Hall). The specific MCMC implementation we use is a *Gibbs sampler*.

The performances of the method will be illustrated on simulated data. It will be shown in particular that cases intractable with current spike sorting methods can be easily dealt with. Strategies to deal with uncertainty on model parameter values will be presented. Our method generates a "soft" classification for each recorded spike, that is, it estimates the probability for each event to have been generated by each neuron in the model. Other methods generate "soft" classification as well (e.g., Lewicki, 1994, *Neural Comp*, 6, 1005) but until now the classification has always been "forced" into the most likely neuron of origin for subsequent analysis, like computing cross-correlograms between

two neurons of the model. We will show how to keep soft clustering and therefore avoid the bias introduced by forcing classification.

WAND - an open workbench for the analysis of neuronal data 1083

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To understand the massive parallel and distributed processing in the brain it seems to be plausible to use multi-electrode approaches. Due to the technological progress in the last years this application has become more and more widespread. Based on the individual requirements and some physical properties of the resulting data sets (size, machine dependent word length, etc.) a broad spectrum of different software tools has been independently of each other developed. As a result it is an tedious effort to integrate and adapt different software routines or tools into a personal workflow. Our goal is to develop and distribute an open analysis tool framework which offers a platform to overcome this drawback. The conceptional design of this workbench for the analysis of neuronal data (WAND) is intended to address certain aspects:

- freely, public available - a graphical, fully customizable user interface - easy extend able - easy integration of existing tools or routines - modular concept which separates between frontend and analysis routines

The development of WAND is intended to overcome a series of technical inconveniences and to easy the use of sophisticated tools in the analysis of massive parallel recorded neuronal responses.

An electronic device that measures series resistance during tevc recording in *Xenopus* oocytes 1084

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Xenopus laevis oocytes are an established expression system for ion channels. The two-electrode voltage-clamp (TEVC) technique is a method heavily used for the investigation of the biophysical properties of expressed ligand gated and voltage gated channels. The currents recorded in *Xenopus* oocytes can be large and range up to tens of microamperes.

TEVC recordings are compromised by two major sources of error:

1. The non-ideal geometry of the cell (space clamp error)
2. The electric resistance Rs of the intracellular structures located between the tip of the recording electrodes and the cell membrane (series resistance error).

Artifacts caused by this series resistance (Rs) deteriorate measurements considerably due to the large membrane currents. They fall into two groups:

1. The DC error caused by the voltage divider formed by the series resistance Rs and the membrane resistance Rm.
2. The dynamic error caused by the lowpass filter formed by the series resistance Rs and the membrane capacity Cm.

Therefore, the measurement of Rs is an important parameter that would help to evaluate the recordings. We have designed an electronic instrument that can be used in conjunction with a standard TEVC amplifier (TURBO TEC series, npi electronic) to measure and display Rs automatically.

The instrument is based on the injection of symmetrical current pulses of 10 microamperes and a few kHz around the holding or resting potential of the cell. The membrane potential deviation that appears on the positive slope of the pulse is proportional to Rs and is measured using high precision sample-and-hold circuits controlled by a timing unit that is synchronized from the injection pulses applied. Rs is displayed on a digital meter with a resolution of 10 Ohms and can be also stored on a computer. Rs measurement can be started manually or through a TTL input. After the measurement the stored Rs value is displayed continuously until the next measurement is started. Therefore, measurement can be automated easily using standard lab software.

The high frequency, as well as the symmetry of the amplitude and duration of the applied pulses is very important in order to avoid DC changes of membrane potential which can lead to channel openings.

During TEVC measurements in oocytes the mean Rs value obtained was 1.46 kOhm (n=227) ranging from 0.28 to 8.05 kOhm. Oocytes with high Rs are not under proper TEVC control and their currents appear significantly altered. In some recordings high Rs values could be lowered by repositioning the current electrode.

This instrument is a useful tool that helps to avoid erroneous recordings and facilitates not only accurate TEVC recordings but also precise Cm measurements in *Xenopus* oocytes (see poster Schmitt et al., this volume).

Experiences from the German-French Pupil Outreach Project "Biological Research in Space" Linked to the Andromède Mission to the International Space Station

1086

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The German-French experiment AQUARIUS-XENOPUS investigated effects of gravity deprivation on vestibular development. It flew on the Andromède mission to the International Space Station ISS in October 2001. It was extended by an educational outreach project at which 16- to 18-years old pupils from Ulm/Germany and Nancy-Tomblaine/France participated. The aim of the French and German scientists and supporting German (DLR) and French (CNES) space agencies was to let pupils actively participate in a biological space experiment. The pupils were introduced into (1) *theoretical aspects* of gravitational biology, microgravity, equilibrium and movement control, and (2) *experimental aspects* in space research and procedures. They got the opportunity to present their project on international meetings (Stockholm 2002, Cape Canaveral 2002). The project lasted 1.5 years. Working meetings in Ulm (June 2001), Nancy (February 2002) and Paris (May 2002) were used to exchange ideas and results between the German and French pupils and their supervisors. - The *theoretical project component* included regular lessons and basic experiments such as the self-sensation of postural disturbances by visual stimuli or the subjective vertical. The aim of this component was to show pupils the importance of gravity for evolution and, in particular, for development of anatomical and physiological stability of organisms. A website was created thanks to France-Telecom: *www.xenope.com*. - The *experimental project component* included the analysis of swimming kinetics in *Xenopus laevis* tadpoles during gravity deprivation. Zero-g swimming was recorded by ESA-astronaut Claudie Haigneré aboard ISS. All pupils were trained to carry out the 1g-ground control at the same protocol. Their analysis revealed (1) that swimming periods were longer and the overall swimming speed mostly reduced under microgravity conditions, and (2) that the accelaration of swimming was higher during straightly oriented trajectories. - This project is a model for advanced scientific education of pupils: it uses running biological experiments. Careful education combined with the pupils' enthusiasm for the experiment handling will lead to significant results. Important aspects for future projects include selection of proper experiments which improve the pupils' motivation. Presentations at international conferences by the pupils are important; there they will meet and speak with personalities such as outstanding scientists or astronauts. We recommend to use simple experiments because motivation will be improved if experiments leave to clear-cut results and pupils get the feeling that their project contribute to the work of the scientists.

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1087 Automated and real-time correction of series-resistance errors during membrane capacitance monitoring in the two-electrode voltage clamp mode using a novel hardware device

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We recently presented an improved approach to monitor membrane capacitance (Cm) in large cells such as *Xenopus laevis* oocytes using the two-electrode voltage-clamp (TEVC) technique. This "paired ramps" approach was suited to monitor Cm conveniently, in real-time, and with high temporal resolution, accuracy and precision (Biophys. J. 82:1345-57, 2002). Its performance was limited only by potential clamp errors due to high series resistance (Rs), a general problem in TEVC experiments. At that time, TEVC methods for fast and precise measurement of Rs were unavailable. We proposed, however, that development of a method for monitoring Rs in parallel with Cm should in principle allow to compute the true Cm even when Rs is high, because the observed Cm error strictly followed the theoretical prediction, approximated by $\varepsilon=2Rs/(Rs+Rm)$, where Rm denotes membrane resistance. Meanwhile, a novel hardware device for Rs monitoring in the TEVC mode exists ("Rs box", NPI electronic, Tamm, Germany). Using this Rs box, we now developed a strategy for the automated correction of Rs errors and implemented it in the hardware and software setting of our previously described Cm monitoring approach. In brief, this is achieved in a "two-stroke cycle": First, the Rs box applies an Rs test pulse, samples and holds the Rs value, then the clamp amplifier (TEC-05, NPI electronic) follows with the Cm test pulse; pulse sequence is controlled by a commercial software (PULSE, Heka, Germany). After each "two-stroke cycle", a second software (X-CHART, Heka) acquires "online analysis" data from PULSE (yielding raw Cm), and the stored Rs value from the Rs box, and uses both for real-time computation of the true Cm according to the indicated formula. Performance of this approach was tested in a calibrated electrical cell model with tunable Rs, Rm, and Cm. Varying the critical ratio Rs/(Rs+Rm) over a wide range at a fixed potential of -50 mV and a Cm of 160 nF was associated with membrane currents between -5 nA and -10 microA. Under these conditions, uncorrected Cm measurements varied between 115-160 nF, underestimating true Cm by up to 30%. After correction of the Rs error, Cm values accurately reflected the true value within 1 nF (<1%). In spite of an increased cycle duration, a high Cm sampling rate of several Hz could be maintained. In *Xenopus* oocytes, Rs measurements were sensitive to clamp artifacts (non-linear electrode response, capacitive coupling, etc.) and required optimization of Rs stimulus parameters and clamp performance. Taken together, we have established a simple and efficient method to obtain valid Cm measurements even in the presence of high Rs. This method extends the application range of Cm monitoring using the "paired ramps" approach to oocytes with overexpressed and activated ion channels. In a wider context, simultaneous measurements of Rs and Cm may prove useful for elucidating in *Xenopus* oocytes the largely unexplored biological underpinnings of these two electrical parameters.

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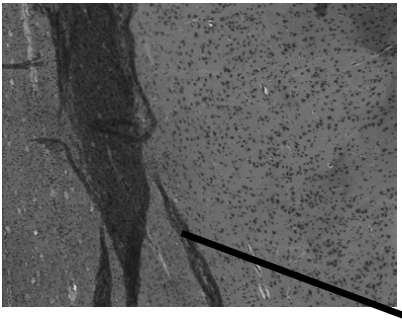

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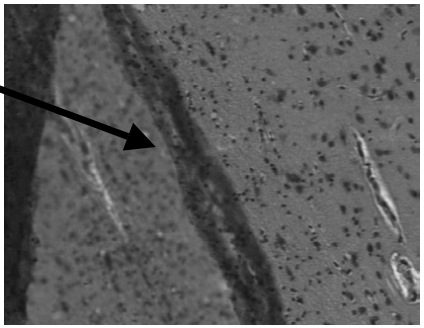

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Introductory Remarks to the Satellite Symposium A:

Inhibition: molecules, mechanisms, functions

Symposium of the Neurocenter, University of Ulm, Germany

Günter Ehret, Joachim Kirsch* and Albert Ludolph

Functions of the nervous systems of animals and humans emerge from the mutual antagonism of excitation and inhibition including modulatory influences of neurons with excitatory and inhibitory net effects. Inhibition may attenuate, filter, and shape excitatory states and excitatory outputs of neurons both in magnitude and timing.

Inhibitory actions can be studied at many levels of neural systems. Starting with the cellular level, inhibitory effects are mediated by receptors of neurotransmitters and neuromodulators and carried out directly by ion-channel activities and indirectly by intracellular signaling cascades. Many genes code, in mostly unknown ways, for subunits of receptors and ion channels, so that it is important to know the differential pattern of differential gene expression in neurons in order to predict functions and malfunctions of inhibition at the cellular level. At the level of neural networks, inhibition is involved, for example, a) in setting thresholds, general levels of activity and the exact timing of excitatory actions of neurons and the whole network, b) in differentially shifting activity to certain neural subpopulations of the neural network and gating the network output pattern via certain pathways, c) in generating oscillations, rhythms and spatial maps of graded activity. At the level of system functions such as sensory processing and perception, the coordination of movements and control of emotions, inhibition becomes most evident whenever it is impaired so that the normal balance between excitation and inhibition is disturbed and perceptional, movement, cognitive and emotional control is out of order, giving rise to abnormal and pathological states.

One goal of this symposium is to sensitize all those who are working on neurons and brains, to consider inhibition in their research as a pervasive strategy of nervous systems evolved to ensure an optimum of function and functional adaptability. The expert speakers of the symposium will present examples of inhibitory regulation and regulation by inhibition from all the levels mentioned above including invertebrate, and vertebrate species (humans inclusive).

*Now, at the University of Heidelberg, Germany

Molecular determinants of inhibitory synapses 1088

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Neurotransmission is mediated by receptor proteins that are concentrated at distinct postsynaptic membrane specializations. The molecular mechanisms, which establish these specialized membrane domains during synaptogenesis and determine their positions, size and packing densities are poorly understood. Receptor-associated proteins are assumed to play an important role in these processes at excitatory as well as inhibitory synapses. The peripheral membrane protein gephyrin was shown to be essential for the formation of glycine- and $GABA_A$ receptor clusters at inhibitory postsynaptic membrane specializations, as lack of gephyrin expression abolishes receptor cluster formation *in vitro* and *in vivo*. The interaction of gephyrin with inhibitory neurotransmitter receptor is thought to reduce the lateral mobilities of receptor proteins thus generating local accumulations. A short sequence motif within the large cytoplasmic loop of the glycine receptor β subunit mediates the interaction with gephyrin, whereas γ_2 subunits seems to mediate the interaction with gephyrin at $GABA_A$ receptors. This interaction seems to be indirectly, however, and involves additional yet unknown components. As gephyrin was shown to bind to polymerizing tubulin and more recently to the light chain of the microtubule motor protein dynein, it is thought that gephyrin binds to subsynaptic microtubules. Moreover recent experiments show that gephyrin binds to and colocalizes with the GDP-exchange factor collybistin, the actin binding protein profilin and the microfilament adaptors Mena/VASP. The latter organize actin filaments into specific suprastructures and can recruit profilin-actin complexes to cellular regions engaged in actin filament dynamics. Hence, profilins and Mena/VASP proteins are both considered essential for submembraneous actin filament generation and organisation. It could be shown that, microtubules and microfilaments seem to act antagonistically on the packing densities of postsynaptic receptor clusters which was shown to alter the affinities of postsynaptic neurotransmitter receptors. Thus, the cytoskeleton is thought to play a pivotal role in regulating the efficacy of inhibitory synaptic transmission.

Chemical and electrical synapses at GABAergic interneurones and significance thereof for synchronous activity 1089

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The fast component of excitatory postsynapic currents (EPSCs) in central neurones is mediated by a glutamate receptor subfamily, the AMPA receptor channels. These receptors assemble from subsets of four subunits - GluR-A to -D. Analysis of single identified neurones in acute brain slices with respect to their electrophysiological and molecular characteristics can reveal the cell-specific use of different genetic mechanisms in the generation of functionally different glutamate receptors. Thus, our studies established that the differential expression of the GluR-B and GluR-D subunit genes account for differences in gating and calcium permeability of native AMPA receptor channels in

GABAergic interneurones. The question as to the functional significance of different AMPA receptors on interneurones at the system level will be discussed. We have approached this problem by generating transgenic mice with altered AMPA receptors in interneurones. Thus, mice overexpressing the GluR-B subunit in GABAergic interneurones or mice lacking the GluR-D subunit should perturb synchronous network activity.

Furthermore, using transgenic approaches we have generated mice in which the *in vivo* marker EGFP is expressed in specific interneurone subpopulations. The easy identification of neuronal subpopulation in the acute slice preparation will help in the functional characterization of native receptors in neurones that are critical in controlling network behaviour.

These mice will also help in establishing the participation of specific GABAergic subpopulations for the generation of synchronous oscillatory activity at specific frequencies. Thus, making use of transgenic mice with *in vivo* labelled parvalbumin-positive interneurones, we have identified a new cell type that is critically involved in the generation of oscillatory activity in the θ frequency range. Furthermore, in these mice it is easy to identify intercellular communication via electrical synapses and establish their contribution for rhythmic oscillatory network activity.

1090 Functional relevance of GABA-A receptor subtypes

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In the harmonious brain, excitatory and inhibitory neurotransmission coexist in a purposeful balance. Excitatory projection neurons link different brain regions. In contrast, neurons that signal GABAergic inhibition are largely local, highly diverse and strikingly different as shown morphologically and electrophysiologically. The target neurons which are under GABAergic inhibitory control can be distinguished by the type of GABA-A receptor expressed in a neuronal ensemble. To define the role of inhibitory neuronal circuits in the regulation of behavior, particular receptor subtypes were genetically modified. They were rendered insensitive to diazepam by the introduction of a point mutation into the drug binding domain of either the α1, α2, α3 or α5 subunit. These subunits characterize different sets of receptor subtypes which display a distinctive pattern of expression in neuronal circuits. By genetically manipulating a receptor subtype, the contribution of the respective neuronal ensemble to the drug-induced behaviour can be determined.

The sedative action of benzodiazepines and most of the anticonvulsant activity was found to be mediated by neurons expressing α1 GABA-A receptors (ref.1). In contrast, the anxiolytic action was attributed to α2 GABA-A receptors which display a high density at the axon initial segment of principle cells in critical brain regions (ref.2). The muscle relaxant activity was multimodal, being mediated by neurons expressing α2, α3 and α5 GABA-A receptor. Most remarkably, in the α5-point mutated mice a slight deficit of α5 GABA-A receptors in hippocampal pyramidal cells was apparent. This cell-specific partial deficit of α5 GABA-A receptors resulted in a drug-independent phenotype. Temporal memory as well as spatial memory formation was enhanced in the

α5-mutant animals (ref.3). Thus, the largely extracellular α5 GABA-A receptors play a major role in hippocampal associative memory formation. These receptors offer new avenues for the development of agents improving memory function. Thus, the genetic dissection of GABAergic inhibitory pathways provided new insight into the neural regulation of emotion and cognition.

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Impaired inhibition as a pathophysiological mechanism in idiopathic epilepsies 1091

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In the recent past, molecular genetic and electrophysiological investigations revealed that the idiopathic epilepsies belong to the increasing group of channelopathies, i.e. inherited disorders caused by mutations in ion channel encoding genes. The molecular pathophysiological mechanisms of some of these mutations in specific syndromes point to a major role of $GABA_A$ receptor-mediated inhibition in idiopathic generalized epilepsies (IGE). Generalized epilepsy with febrile seizures plus (GEFS+) and severe myoclonic epilepsy of infancy (SMEI) are caused by mutations in subunits of the voltage-gated Na^+ channel or the $GABA_A$ receptor (SCN1B, SCN1A, SCN2A, GABRG2), and mutations in GABRG2 and GABRA1 were also found in families with classical forms of IGE such as juvenile myoclonic epilepsy (JME) or childhood absence epilepsy (CAE). Whereas for Na^+ channel mutations associated with GEFS+ both gain- and loss-of-function mechanisms were described recently, mutations in $GABA_A$ receptors and the SMEI-causing Na^+ channel mutations lead to a loss-of-function. In different classical forms of IGE, mutations in the Cl^- channel CLC-2 were found, which can induce neuronal intracellular Cl^- accumulation and/or depolarization of the postsynaptic membrane in GABAergic synapses. The majority of these effects, including the loss-of-function of Na^+ channels, probably results in dysfunction of GABAergic inhibition which might evolve as a general concept in different forms of IGE.

Inhibition of muscles makes them move faster: Arthropod common inhibitors 1092

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Inhibitory motor neurones in arthropods are a particularly interesting group of neurones for several reasons. They may be identified as individual cells and thus lend themselves to comparative analyses among arthropods and also, under a functional perspective, for comparison with vertebrate motor systems. Their morphological and functional modifi-

cation during arthropod evolution may shed light on phylogenetic relationships of arthropod groups.

In arthropods, supply of limb and body wall muscles by inhibitory motor neurones is a common feature (reviews in Wiens 1989, J Neurobiol 20: 458-469; Rathmayer 1998, Leopoldina (R.3) 43: 221-235). Arthropod muscle is innervated by a small number of motor neurones, usually between three and just over ten, and is composed of relatively few muscle fibers, often less than 100. These features make inhibitory innervation an essential element of arthropod motor control strategy, and require radical differences from the strategy used by vertebrates to allow fine control of muscle contraction with regard to both, force output and contraction kinetics.

In a given muscle, motor neurones supply overlapping motor units, with heterogeneous fiber composition. This allows the differential recruitment of muscle fibers according to particular motor tasks. However, it would also lead to the recruitment of slow muscle fibers during rapid and powerful movements, in addition to their recruitment in the context of posture control. The resulting build-up of residual muscle tension, due to slow contraction and relaxation kinetics of slow muscle fibers, would be particularly obstructive during rapid alternating movements, for instance, escape running. These effects are avoided by the inhibition of slow muscle fibers during rapid movements through motor neurones of the common inhibitory (CI) type, which supply most or all muscles of an appendage.

This basic pattern of CI function was modified during arthropod evolution in several ways. For example, specific inhibitors in decapod crustaceans are used to uncouple muscles with shared excitatory motor neurone supply.

1093 Glycinergic inhibition in audition: Specific functions in temporal processing

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Inhibition has long been neglected as a major player in sensory processing. It has rather been viewed as modulating excitatory inputs or as being involved in gain control, adjusting the presumably more specific excitatory interactions. However, this view has been in progressive contradiction to an increasing number of anatomical findings that suggest structural specializations indicating very fast and very precise synaptic inhibition to or from inhibitory neurons. Recently, strong evidence emerged that inhibitory projections can be functionally very specific, shaping firing patterns of single neurons in response to sensory stimuli with a so far unknown precision (1,2), or, for instance, narrowing the time windows for coincidence detection in the hippocampus (3).

Our recent recordings from the mammalian auditory system show that inhibitory input can sculpture response patterns in the millisecond and adjust processing of binaural information even in the sub-millisecond range. Studies in the bat auditory midbrain proved well-timed GABAergic and glycinergic inhibition to be essential for creating neuronal selectivity to the sound duration (4). In the bat auditory brainstem, the exact

temporal interaction of excitatory and inhibitory inputs driven by the same ear create a selectivity to pause durations in the range of a few milliseconds (5), or can set filters cut-offs for amplitude modulations at some hundred Hertz. The most precise inhibition, however, emerges from the medial nucleus of the trapezoid body. This glycinergic projection has been shown to be responsible for the adjustment of the sensitivity of medial superior olive neurons to interaural time differences (ITD), although these ITDs are in the range of only microseconds (2). Hence, inhibition serves very specific and elaborate functions in the context of the temporally most precise task the mammalian brain can perform. Interestingly, this adjustment by inhibition is shaped during development and depends on activity and experience of spatially meaningful acoustic cues.

These findings establish inhibition as a specific and important factor in temporally precise computation.

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Extra-classical receptive field responses – Balanced inhibition and excitation in visual Gestalt organization 1094

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Recent investigations revealed that the generation of neural representation of visual surface layout in cortical area V1 utilizes information from spatial regions outside the classical receptive field (CRF). For example, the response of oriented cells probed by an optimally oriented bar was reduced when the same bar was displayed as an item of a texture of bars of random orientation. Responses were raised again – sometimes even exceeding the initial activity level – when the initial bar at the target cell was supplied by like-oriented bars presented co-axially. Such evidence supports the view that Gestalt grouping into smooth boundaries is implemented early in the visual pathway. Co-aligned bars mutually support each other while the surround items of random orientation inhibit each other.

In motion perception, a moving bar is analyzed by direction-sensitive V1 cells. However, these mechanisms suffer from the aperture problem of motion detection. Cell responses at the center of a diagonally moving vertical bar have been demonstrated to initially (within 60ms) generate maximal responses in a direction orthogonal to local contrast. Later (after 100ms) the maximum selectivity is shifted towards the true diagonal movement direction. The uncertainty is resolved by means of either lateral long-range interactions or feedback from higher areas such as MT. Again, such interactions involve excitatory as well as inhibitory mechanisms in order to generate perceptually unambiguous representations.

Our modeling investigations demonstrate that excitation is balanced by inhibition such that enhancement of activations and suppression of activity is mediated by neural interactions beyond the CRF. Such an interplay is responsible for the generation of visual pop-out and the disambiguation of motion patterns. Withdrawal of inhibition leads to

undesired saturation of activity causing a loss of differentiation and localized representation. In all, inhibition functions as a significant regulator for the generation of representations of salient visual structure and layout.

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1095 Inhibition and the prefrontal cortex: A central mechanism for cognitive and emotional control

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Starting from ideas developed by Miller and Cohen (2001) in a detailed review of frontal lobe functions within the context of neural network modeling of cognitive control, several general features are outlined. The different parts of the prefrontal cortex subserve similar functions of inhibition, context updating, and planning. These functions can be applied to different aspects of high-level information processing. For example, whereas in the dorsolateral prefrontal cortex abstract rules of language (left) and visuospatial thinking are processed and (therefore in the long term) represented, the orbitofrontal cortex deals with the output of systems related to reward and motivation, and hence, comes to represent goals, emotive experiences, and values in the long run. Neuroimaging studies of prefrontal cortical functioning are reviewed to support this parsimonious view. In particular, the function of the prefrontal cortex of generating and storing rules is scrutinized from a systems neuroscience point of view. Studies conducted in Ulm on various aspects of frontal lobe functioning are presented. The concept of hypofrontality in psychiatric disorders is criticized for its simplicity. Data from neuroimaging studies in patient groups do not support the notion of a general frontal deficit in schizophrenia and depression.

Miller EK, Cohen JD (2001) An integrative theory of prefrontal cortex function. Annual Review of Neuroscience 24: 167-202.

1096 Critical bandwidths and inhibition in auditory midbrain neurons of house mice

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Critical bandwidths (CBs) are well-known psychophysical phenomena describing perceptual mechanisms of sound frequency resolution. Studies on the neurophysiological basis of CBs showed that their main properties are coded in the auditory midbrain inferior colliculus (Ehret and Merzenich (1985) Science 227: 1245-1247; Vartanyan et al. (1999) Dokl.Biol.Sci. 368: 437-439). Analysis of the shape of the excitatory and inhibi-

tory receptive fields of inferior colliculus neurons in relation to neural CBs raised the hypothesis that inhibition is a main neurophysiological mechanism of critical bandwidth regulation (Ehret and Merzenich (1988) Brain Res.Revs. 13: 139-163). To clarify the role of inhibitory processes in forming neural CBs, the parameters of neural CBs and inhibition were analyzed and compared in mouse inferior colliculus neurons. In 45 anesthetized (ketamine/xylazine) female house mice (strain NMRI), neural CBs were estimated with a narrowband-noise masking procedure and excitatory and inhibitory receptive fields of 103 neurons were mapped by single tones and two-tone complexes. Measurements were taken within the whole frequency range of hearing (3-80 kHz) and sound levels from units' thresholds up to 80 dB above it (from -20 dB SPL to 90 dB SPL). As defined before by shapes of excitatory and inhibitory receptive fields (Vartanyan et al. (2000) Dokl.Biol.Sci. 373: 364-366; Egorova et al. (2001) Exp.Brain Res. 140: 145-161), four classes of neurons were found: primary-like, inhibition-dominated, V-shaped, and complex. Analysis of the relationship between the low- and high-frequency borders of neural CBs and the neurons' excitatory characteristic frequencies (CFE) showed that the CB is not centered at units' CFE. The average distance between CFE and CB borders varied depending on the class of neurons from 1/12 up to 1/6 octaves for the high-frequency CB border and from 1/6 up to 1/3 octaves for the low-frequency CB border. Comparing inhibitory receptive fields with CBs showed highly significant correlations between the best frequencies of inhibition below and above the CFE and respective CB low- and high-frequency borders in each class of neurons. This indicates that perceptual CBs are reflected by inhibitory receptive fields of neurons in the inferior colliculus.

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Inhibitory motor neurones in the abdomen of locusts, stick insects and dragonflies are putative homologs 1097

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Inhibitory motor neurones in the abdominal segments of locusts, dragonflies, and stick insects were identified morpholically, and compared to reveal possible homologies. A specific goal was the explanation of peculiarities observed in recent analyses of the inhibitory motor neurone supply in locust body wall muscles, for example, variable numbers of inhibitory motor neurones per segment, and presence of specific, as opposed to common, inhibitors (Schmäh, Bräunig & Wolf, this volume; Schmäh & Wolf, 2003, J Exp Biol 206: 445-455).

Inhibitory motor neurones are not only a unique group of neurones (Wolf, this volume) but they are particularly well-suited for comparative analyses because they are easily identified. This is done by combining motor nerve backfills (e.g. with neurobiotin) with immunocytochemistry directed against the inhibitory transmitter of insect motor neurones, γ-amino-butyric acid (GABA).

All three insect groups - locusts, stick insects, and dragonflies - possess two (putative) inhibitory motor neurones in each of their unfused abdominal ganglia. They exhibit morphological characteristics of body wall inhibitors CI_a and CI_b described recently in the locust (Schmäh & Wolf ibid): They have cell bodies located posteriorly and contralaterally in the ventral soma cortex, conspicuous looping primary neurites, and bilateral arborisations in the dorsal (motor) neuropil. In dragonflies, there is a third inhibitor with similar characteristics.

On the grounds of morphological data we postulate homology of the two neurones in the stick insect abdominal ganglion, and of two of the three neurones in the dragonfly abdominal ganglion, with locust CI_a and CI_b, innervating dorsal and ventral body wall muscles, respectively. The third neurone observed in the abdominal ganglia of the dragonfly may be homologous to specific inhibitor SI_{60} in the locust prothorax (Schmäh, Wolf & Bräunig ibid), a segment also possessing three body wall inhibitors (CI_a, CI_b, SI_{60}). The presence of three body wall inhibitors per segment may reflect an ancient (plesiomorphic) situation, possibly the insect ground pattern.

Capture and use of dragonflies was granted by Höhere Naturschutzbehörde, Regierungspräsidium Tübingen (Az. 56-6/8852.15-41).

1098 Specific inhibitory motor neurones supply body wall muscles in the locust prothorax

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Specific inhibitory motor neurones (SIs) which supply a particular muscle were hitherto thought to be a peculiarity of decapod crustaceans. In their distal leg segments, specific inhibition is necessary to uncouple a pair of leg muscles with shared excitatory innervation (review in Wiens 1989, J Neurobiol 20: 458-469). This situation probably arose in the course of limb evolution by the division of a single muscle into two portions, subserving adjacent leg joints.

Here we demonstrate that insects also possess specific inhibitory motor neurones. We combined labeling of motor neurons by peripheral nerve backfills, immunocytochemistry directed against the inhibitory transmitter γ-aminobutyric acid, and extra- and intracellular electrophysiology to identify inhibitory motor neurones in the prothoracic ganglion of the locust, *Locusta migratoria* L..

In addition to the well-studied common inhibitory motor neurones which innervate all muscles of the prothoracic leg, CI_{1-3}, there are three inhibitory motor neurones which leave the prothoracic ganglion via nerve 6, and supply body wall muscles. Their morphology in the ganglion conforms to that of CIs recently described in the locust abdomen (Schmäh & Wolf, 2003, J Exp Biol 206: 445-455), with cell bodies located contralateral to the nerve of exit, posterior and ventral in the ganglion, and with looping primary neurites and bilateral dorsal arborisations. Two of the inhibitors innervate mu-

scles 59/60 and 59/81/82, respectively, and thus are common inhibitors, the third cell specifically supplies muscle 60, and thus is a specific inhibitory motor neurone.

Similar to the situation in the decapod crustacean leg, these specific inhibitors appear to have evolved from common inhibitors by restriction of their innervation area, or by loss of innervation targets.

'Inhibition' participates in endogenous and exogenous neuroprotection against ischemic damage

1099

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Excitotoxic activation of glutamate receptors is thought to play a crucial role in triggering neuronal death both after global and focal cerebral ischemia. Neurons at risk can be protected against ischemic damage by induction of an endogenous ischemia tolerance with a short sublethal priming stimulus, e.g. a short ischemic period ("ischemic preconditioning"). Neuronal death can also be prevented by exogenous application of a battery of neurotrophic factors. The exact molecular basis of these different neuroprotective strategies is still unclear. Recently, we could demonstrate by quantitative receptor autoradiography that ischemic preconditioning is associated with increased ligand binding of [3H]muscimol to inhibitory $GABA_A$ receptors in the hippocampus [1]. These findings suggest that a shift between inhibitory and excitatory neurotransmission in the hippocampus may participate in the induction of an endogenous tolerance against neuronal death after global ischemia.

Recently, we could demonstrate that exogenous application of brain-derived neurotrophic factor (BDNF) in a rat model of focal cerebral ischemia significantly reduced infarct volumes in the cortical penumbra [2]. Since BDNF is known to modulate expression and function of various neurotransmitter receptors, we put forward the hypothesis that modifications of postischemic ligand binding to inhibitory GABAA receptors may also participate in the neuroprotection observed. Transient focal cerebral ischemia was induced in adult male Wistar rats for 2 hrs using the suture occlusion technique. BDNF (300µg/kg per hour) was continuously infused for 3 hrs starting 30 min after ischemia induction. Two experimental groups were investigated: BDNF (n=5) and placebo treated rats (n=5). Using quantitative receptor autoradiography, postischemic ligand binding of [^{3}H]MK-801, [^{3}H]AMPA and [^{3}H]muscimol to excitatory glutamate and inhibitory GABAA receptors, respectively, was analyzed in the ischemic core, cortical penumbra and corresponding contralateral regions. Ischemia caused a significant reduction of [^{3}H]muscimol binding to $GABA_A$ receptors within the cortical penumbra of placebo-treated rats. This was prevented by application of BDNF. To exclude, that this effect of BDNF on [3H]muscimol binding is only due to the smaller infarct sizes in the BDNF treated group, a correlation analysis was performed. Using Spearman´s correlation, a

correlation coefficient r_s of -0.31 (2-tailed, p 0.4142) was computed, indicating that our observed effect is specific and does not depend on infarct volumes. No differences of [^{3}H]MK-801 and [^{3}H]AMPA binding values were seen between placebo and BDNF treated rats. We suggest, that the neuroprotective effect of BDNF in the cortical penumbra is mediated in part by a relative shift between excitatory and inhibitory neurotransmission.

Currently, we are investigating whether the neuroprotection achieved by application of granulocyte-colony stimulating factor (G-CSF) after focal cerebral ischemia is also accompanied by maintained [^{3}H]muscimol binding in the cortical penumbra, which would suggest activation of the GABAergic system as a more general neuroprotective mechanism after cerebral ischemia.

References:
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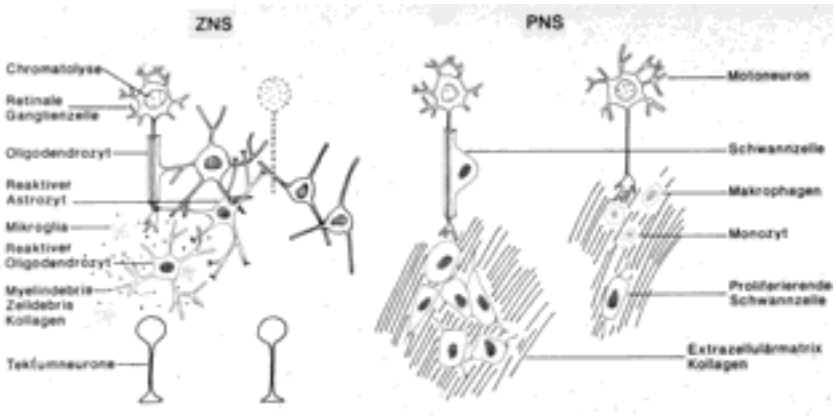
Introductory Remarks to Satellite Symposium B:

Molecular basis of neural repair mechanisms

Mathias Bähr and Hans-Werner Müller

The adult central nervous system (CNS) of mammals possesses only little ability for self-repair after an injury, that is, most parts of the CNS cannot generate new neurons and do not regenerate axons. Therefore, repair of damaged functional circuits is severely limited. This is in contrast to the peripheral nervous system (PNS) or the immature mammalian CNS, where a successful regeneration is possible. At present no therapies are available that can be applied to human patients. However, from a basic science perspective many recent advances in this field have been made, which provide a solid foundation for progress towards the development of effective treatments. The apparent lack of the adult mammalian CNS to regenerate occurs albeit the inherent ability of CNS axons to re-initiate axon growth at least to some extent. Work of the last decades has led to the characterization of factors associated with this inability such as lack of growth-encouraging factors, the inability to express the full set of molecules required for outgrowth and guidance, scar formation at the site of injury, the presence of growth inhibitory molecules, and also the degeneration of axotomized neurons which will be discussed further in this symposium. At present, various approaches are being investigated aimed at overcoming these obstacles, including the use of neutralizing monoclonal antibodies against growth-inhibiting activities, interference with signalling pathways activated by inhibitory molecules, prevention/removal of the scar tissue, blocking of apoptosis, implantation of growth-promoting or stem cells or the expression of growth promoting proteins via several routes including vector based strategies.
With an increased understanding of the factors contributing to the inhibition of regeneration and therapeutically targeting, the possibility arises that finally regeneration of axons and topographically correct re-innervation of their target tissue may be achieved.



Neuroprotection by the inhibition of apoptosis 1100

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Abstract has not been submitted

Molecular switches in neuronal cell death 1101

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Abstract has not been submitted

Neuroprotection by ischemic preconditioning 1102

Ulrich Dirnagl

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Abstract has not been submitted

Role of Inhibitory Apoptosis Proteins (IAPs)in Neurodegeneration and Disease 1103

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Abstract has not been submitted

Slits and semaphorins, not just axon guidance molecules 1104

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Abstract has not been submitted.

1105 Reggie and Nogo functions in neurite growth

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Abstract has not been submitted

1106 Chemorepulsive semaphorins in neuroregeneration

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Abstract has not been submitted

1107 The role of Proteoglycans in regeneration and plasticity

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Abstract has not been submitted

1108 Tenascin-C and related ligands in CNS wound reaction and repair

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Abstract has not been submitted

1109 Olfactory ensheating glia autotransplantation: A therapy to repair injured spinal cords in primates

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Abstract has not been submitted

Axon regeneration through the mature optic nerve 1110

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Abstract has not been submitted.

Toward a stem cell therapy for Parkinson's disease 1111

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Abstract has not been submitted.

ES cell-based neural transplantation 1112

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Abstract has not been submitted

Brain repair in experimental and clinical Parkinson's disease 1113

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Abstract has not been submitted.

Optimization of viral vectors for neurodegenerative diseases 1114

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Abstract has not been submitted.

1115 The potential of lentiviral vectors for neurodegenerative diseases

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Abstract has not been submitted

1116 The role of training and experience in graft-derived recovery of function

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Abstract has not been submitted

Introductory Remarks to the Satellite Symposium C:

2. International transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) Symposium Göttingen

W. Paulus, F. Tergau, M. Nitsche and U. Ziemann

The interaction of human brain function with artificially induced intrinsic brain electricity is the central topic of this symposium. Short electric currents in the brain can be induced pain free by pulsed transcranial magnetic stimulation (TMS). With TMS applied in a repetitive mode (rTMS) succeeding pulses interact and may induce excitability alterations outlasting the stimulus train. Finally, transcranial direct current stimulation (tDCS) can directly modulate membrane polarisation and firing rates of cortical neurones. This symposium updates the knowledge of brain function gained by TMS and tDCS since the introduction of TMS in 1985. It was designed as a follow-up meeting of a first symposium held in Göttingen in 1998 and expands to recently developed areas of neuroimaging, neuropsychology and neural plasticity research using these techniques. TMS now has a definite place in neurological diagnostics in order to quantify alterations of conduction velocity or axonal loss of the pyramidal tract. More selective stimulation techniques in terms of coil design and pulse shape are currently being developed. tDCS has regained interest in recent years after it was shown that it definitely modulates cortical excitability. rTMS and tDCS after-effects can be shaped with concurrent drug applications. Several paired stimulation techniques allow detection of after-effects lasting 24 hours and longer.

In addition, electric stimulation of the brain may be used as a therapeutic tool in neuropsychiatric diseases. An already established therapeutic application of electric stimulation is deep brain stimulation in Parkinson's disease or dystonia. Non-invasive stimulation techniques would avoid invasive surgery and are approached in future as experimental therapeutic research. So far progress has been made in using rTMS in the treatment of depression, whereas the use of rTMS in other diseases such as epilepsy or movement disorders is still experimental. Technical innovations are a prerequisite for the biological progress of this field. Interactive discussions about techniques, their applications and objectives are expected in order to move this research forward.

Eighteen Years of TMS - Principles and Practice 1117

Anthony Trevor Barker

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Transcranial magnetic stimulation was first demonstrated in 1985 and interest in it has grown rapidly since then. Today several thousand stimulators are in use world-wide in basic research, diagnosis and therapeutic applications This paper sets the scene for the Goettingen 2003 International TMS Symposium by charting the progress of magnetic stimulation, from the discovery of the underlying scientific principles in the nineteenth century, up to the present day. Magnetic stimulation is based upon the principle of electromagnetic induction discovered by Michael Faraday in 1831. It uses a brief but intense magnetic field pulse to induce currents within the body. If these currents are of appropriate amplitude, duration and orientation they will stimulate excitable structures, in just the same way as currents injected into the body via needle or surface electrodes.

Magnetic stimulation has a major advantage over conventional electrical stimulation when used to stimulate the human brain, namely the magnetic field passes through the skull without attenuation. This results in discomfort-free stimulation, enabling the technique to be readily used on both patients and volunteers alike.

Continuing technical advances have addressed some of the limitations of the early hardware, such as stimulus repetition rate, and modern stimulators which can run at tens of stimuli per second for short periods are now widely used. However, there is scope for more development, particularly in the areas of coil heating, electrical efficiency and the use of ferromagnetic materials in coil construction.

The basic physics of magnetic stimulation appears to preclude the holy grail of selective stimulation of a small volume of tissue at depth. However, new applications of rapid rate stimulation, for example in psychiatric disorders, muscle rehabilitation, functional stimulation, pain relief and other therapies, are likely to lead to continuing interest in the technique for many years to come.

Contributions to the field by Bernd-Ulrich Meyer and Simone Röricht 1118

Stephan A. Brandt, Martin Voss, Andrea Kühn and Kerstin Irlbacher

Dept. of Neurology, Charité, Augustenburgerplatz 1, 13348 Berlin, Germany

This presentation will be given to honour two outstanding scientist for their contribution to clinical neurophysiology and system neurophysiology. Bernd-Ulrich Meyer and Simone Röricht most untimely died with their children in the CrossAir flight accident near Zürich on Saturday, November 24, 2001.

Bernd-Ulrich Meyer was first introduced to the field by Prof. R Benecke. Immediately fascinated by this new technique he investigated basic mechanism of TMS of the brain and cranial nerves, and later the application of TMS in the diagnosis of facial paresis, assessment of intracortical excitatory and inhibitory interneuronal action, reorganisation

of the corticospinal system after hemispherectomy and changes of cortical excitability in basal ganglia diseases. His aim was to examine a variety of different motor disorders with TMS to gain insights into physiological and pathophysiological mechanisms of the motor system. During 1988 and 1991 Bernd-Ulrich Meyer published more than 20 investigations often co-authored by Prof. R Benecke and Prof. Conrad. Still during his appointment in Düsseldorf he met his wife Simone Röricht, who worked on her doctoral thesis about the analysis of single motor neuron firing after TMS in patients with MS. Together they moved to Munich and focused on studying the assessment of peripheral nerve lesions by TMS at the Dept. of Neurology (Prof. Conrad). During several months in 1992 with John Rothwell at the MRC Human Movement and Balance Unit in London they also met Dr. K. Werhahn and together they investigated corticospinal excitability of individual motorneurons and the effects of cerebellar stimulation on motor cortex excitability. In 1992, Bernd-Ulrich Meyer published the well known first German handbook on TMS, giving an overview on anatomical, functional, technical and diagnostic aspects of TMS.

Later, from 1994 until their death Bernd and Simone worked together in tight scientific co-operation at the Dept. of Neurology at the Charité hospital in Berlin. They co-authored nearly 100 papers, often highly original and always carefully-designed and meticulously-conducted studies. The most important contributions were about the cerebrovascular reaction to TMS, the assessment of corpus callosum functions in normal subjects and patients with lesions, brain plasticity and visual system and higher cognitive functions such as working memory using transcranial magnetic stimulation. Simone has provided novel and important insights into the neural plasticity induced by upper limb amputations, phantom pain, and hand replantations (see contribution by Irlbacher at this symposium). Most recent projects of Bernd-Ulrich Meyer and Simone Röricht included combined transcranial electrical stimulation with fMRI and examination of higher motor functions with both psychophysical and electrophysiological techniques.

1119 Modelling of the stimulating field generation in TMS

Jarmo Ruohonen

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With the increasing importance of magnetic stimulation in investigating the functions and dysfunctions of the brain, it is more and more important to consider and perhaps revisit the basic phenomena behind the method. This presentation includes a layman introduction to the basic physical principles. The presentation will include examples of the effects of coil positioning and orientation to which areas magnetic stimulation presumably touches in the brain. As the second main topic, this presentation will give a short introduction to how frameless stereotaxy can be used to improve the targeting of the stimulation and how the coils may be designed further, e.g., to obtain placebo stimulation randomly interleaved with normal stimuli.

Comparing Coil Caracteristics 1120

Thomas Weyh and Kerstin Wendicke

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OBJECTIVES: A newly for rTMS optimized figure-of-eight coil is compared with two commercially available coils by means of thermal and focal properties.

METHODS: The three coils - *Magstim* coil, *Dantec* coil, and a custom-made coil with a resistance-optimized conductor geometry - were attached to the same energy source (MagPro Stimulator). Heating properties of the coils were measured during *in vitro* rTMS (50-pulse trains of biphasic rTMS at 40-60% of maximum stimulator output). A heating-up curve was obtained, which relates the coil temperature to the number of stimuli applied. The effectiveness of each coil was evaluated *in vivo* by determining the motor threshold (MT) of the right first dorsal interosseus muscle in eleven healthy volunteers and by means of numerical field calculation (focality).

RESULTS: The heating-up curve of the newly designed coil was markedly flattened compared with the conventional coils, resulting in an increase of the total number of stimuli that could be applied by a factor of two and more. The resting MT of the newly designed coil were slightly higher than the *Dantec* coil (mean difference: 2--4% of maximum stimulator output). No differences between the two coils were found for active MT. Both active and resting MT were considerably higher for the *Magstim* coil compared with the other coils (mean differences: 10-15% of maximum stimulator output). Based on these data, it was possible to compute a simulation which accurately predicted the thermal behavior of each coil.

CONCLUSIONS: With our newly developed figure-of-eight coil we could show that the thermal properties of rTMS-coils can be markedly improved without reducing the effectiveness of stimulation.

The triple stimulation technique (TST) 1121

Michel R. Magistris [1] and Kai M. Rösler [2]

[1] Geneva, Switzerland; [2] Berne, Switzerland

The size of motor evoked potentials (MEP) varies markedly among normal subjects and from one stimulus to the next, leading to a broad range of normal values. Furthermore, in normal subjects MEPs are smaller in amplitude than the responses evoked by stimuli performed on the peripheral nerve. As a consequence, the standard MEPs do not allow for a reliable size measurement of the response. Therefore, MEPs are imprecise to disclose failures of cortico-spinal conduction in central nervous disorders.

The triple stimulation technique (TST) links central to peripheral conductions through two collisions. Three stimuli are given, the first is applied to the scalp overlying the motor cortex. After a delay, a second maximal stimulus is applied distally on the nerve supplying the target muscle. The delay is chosen so that the action potentials descending from the cortex collide with the antidromic action potentials evoked distally, with the collision occurring just above the second stimulation site. After another delay, a third

stimulus is applied as proximally as possible to the α cells. This delay is chosen so that ascending antidromic action potentials evoked by the distal stimulus collide slightly distal to third stimulus site. This TST "test curve" is compared to a "control curve" in which the cortical stimulus is replaced by a stimulus applied at the proximal site. The percentage of excited motor axons is calculated from the amplitude ratio of the curves TST test : TST control.

As a result of the collisions obtained by the three stimuli, the action potentials evoked by transcranial stimulation are resynchronized. The phase cancellation occurring between individual motor unit potentials that causes a reduction of the MEP size is thus avoided. Thereby a quantification of the proportion of motor units activated by the transcranial stimulus becomes possible. With sufficient stimulus intensity and facilitation manoeuvres, the TST demonstrates excitation of nearly 100% of the spinal motor neurones supplying the target muscle in healthy subjects.

The TST has demonstrated that 1) the relative small size of MEPs is due to phase cancellation, caused by the desynchronization that occurs within the cortico-spinal tract, or at spinal cell level, or both; 2) the changing synchronization of the action potentials evoked by transcranial stimuli contributes importantly to the variability of MEPs.

The TST has provided new insights into the normal behaviour of cortico-spinal tract conduction. It improves the standard MEPs. It allows a precise detection and quantification of central motor conduction failures due to inexcitability or loss of cortical motor neurones, to conduction block or lesion of cortico-spinal axons. The TST is useful to follow the course and the effects of treatments in disorders affecting central motor conduction. Clinical applications will be elaborated further in the presentation by Rösler and Magistris.

1122 EEG responses to TMS

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TMS combined with EEG allows direct measurement the local excitability of and area-to-area functional connectivity of the cortex (Ilmoniemi et al., 1997). With this technique, we have measured how the transcallosal effects of TMS depend on the precise site of stimulation of the sensorimotor cortex. (Komssi et al., 2002). Although the interhemispheric activation times (about 20 ms) were quite uniform, the pattern of contralateral activation depended strongly on the location of the coil, indicating high specificity in the stimulation of transcallosal tracts. Contralateral activation has been found also as a response to the stimulation of the left prefrontal (Kähkönen et al. 2001) as well as the occipital cortex (Ilmoniemi et al. 1997). It is noteworthy that the EEG responses to TMS can be obtained at stimulus intensities that are far below the motor threshold (Komssi et al. and Kähkönen et al., in preparation). Furthermore, it has been demonstrated in a study where the motor cortex was stimulated (Kähkönen et al. 2001b) that ethanol (0.8 g/kg) can change the TMS-evoked potentials (peaking 43 ms poststimulus) over the right frontal areas. This is an indication that combined TMS and EEG may reveal changes in functional connectivity when the brain is chemically manipulated.

A direct measurement of a modulatory effect on brain activity by TMS was obtained by Schürmann et al. (2001) who stimulated the left sensorimotor cortex while somatosensory stimuli were given to the right wrist; a TMS-induced enhancement of the P25 component was observed in this study.

Suggestions for the use TMS–EEG in new ways include that of Nikulin et al. (in preparation), who proposed that the TMS-evoked component N100 reflects inhibition and could be used as a measure to map the excitation state of the cortex. This component, when measured from the motor cortex, was found to be reduced markedly when the subject was about to perform a visually timed finger movement. Another new proposal comes from Komssi et al. (in preparation), who modeled the TMS intensity–EEG amplitude relationship. They conclude that the measured intensity–amplitude curve can reveal significant information about the distribution of membrane potentials in the area under study.

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Bipolar versus monopolar transcranial magnetic stimulation 1123

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Introduction: The potential of repetitive transcranial magnetic stimulation (rTMS) to modulate human corticospinal tract excitability has motivated therapeutic trials in neurology and psychiatry. The results of these trials were highly variable, ranging from positive clinical effects to ineffectiveness. We hypothesized that the effects of monophasic and biphasic rTMS on corticospinal excitability might differ from each other, and explored the influence of pulse configuration and current direction on low-frequency and high-frequency rTMS in healthy humans. *Methods:* We used 900 suprathreshold pulses at 1 Hz stimulation in one experiment, and 80 suprathreshold pulses of 5 Hz frequency in another. Stimuli were applied over the optimal abductor digiti minimi representation of the dominant hand in healthy subjects. MEP amplitudes of suprathreshold pulses before, during and after rTMS controlled for rTMS efficacy. *Results:* Both studies confirmed a profound effect of pulse configuration and current direction on rTMS efficacy. For the 1 Hz trial, a lasting corticospinal inhibition was significantly stronger after monophasic than after biphasic rTMS (MEP amplitude reduced by 35±20 % vs. 12±37 %, mean ±SD, ANOVA, effect of type, $p=0.032$, effect of bin, $p=0.013$, no interaction). During rTMS, both stimulus configurations yielded a similar MEP inhibition. In addition, resting motor threshold was higher with monophasic TMS (75.9±13.2% of stimulator output) than with biphasic rTMS (38.8±6.7%, t-test, $p<0.0001$). Details on current direction and on the 5 Hz trial will be presented at the

meeting. *Discussion:* Pulse configuration and current direction need to be taken into account to optimise rTMS efficacy.

1124 TMS and Threshold Hunting

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Standardisation of the stimulus strength is an important problem for the application of transcranial magnetic stimulation. Usually, the strength is given as a percentage of a threshold relevant for the particular brain area (e.g. phosphene threshold (PT) for the visual cortex or resting motor threshold (RMT) for the motor cortex). The threshold strength may be defined as the stimulus strength that evokes some positive response (perception of a phosphene for PT or a motor evoked potential in a target muscle with an amplitude greater or equal 50µV for RMT) with a probability of 0.5 being equivalent to the observation of 50% of positive responses in an infinite number of trials. When estimating a threshold in a concrete experiment two problems arise: the true threshold value is almost certainly at a stimulus strength that is not realisable with current TMS equipment (i.e. somewhere between two values that may be applied by the stimulator) and the number of stimuli cannot be infinite. Most researchers estimate the threshold with a standard procedure as the physically realisable stimulus strength at which 5 positive responses are obtained in 10 trials. This threshold estimation strategy may be quite time consuming and despite the fact that the number of stimuli necessary to estimate a single threshold may approach 100 or more, the estimated value may still be in error. There are alternatives to the usual threshold estimation procedure that were mainly developed in a psychophysical context and have been successfully applied in threshold estimation experiments with electrical stimulation of peripheral nerve fibres. In this contribution I will give an overview of threshold estimation strategies for TMS that are more rapid and are at least as exact as the standard procedure.

1125 Repetitive and single TMS studies in somatosensory system

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In the field of human neuroscience, TMS has been widely used as a new and powerful tool to investigate the local brain function by “interference” approach, which may be achieved by either interrupting or facilitating the cortical activity in question. Two lines of methodologies have been proven to be extremely fruitful; ”on-line” and “off-line” approaches. On-line approach is to interfere with the cortical information processing by stimulating the relevant cortical region during the task performance, so as to altar the behavioral outcome. Off-line one is to utilize the carrying-over effects of rTMS that outlast the period of stimulation, to change the behavioral outcome. In this presentation,

comparison between these two methodologies will be highlighted in the examples of somatosensory research.

Here, we investigated the human somatosensory function by using TMS (both on-line and off-line methods), extending previous magnetoencephalographic studies which suggested the hierarchical organization of primary and second somatosensory areas (S1 and S2) for the somatosensory perception and cognition (Mima et al, 1998, Fujiwara et al., 2002). In the first experiment, the effect of low-frequency rTMS (0.9 Hz, 15 min) of the primary sensorimotor area (SM1) was tested by psychophysiological measures. Tactile threshold of the hand contralateral to the stimulated brain was increased after rTMS (Satow et al., 2003).

In the second experiment, the functional role of S1 and S2 was investigated by testing whether the single TMS can transiently modify the somatosensory cognition during the somatosensory stimulation intensity discrimination task. TMS was given at S1 or S2 with the variable delays of 20-200 ms following the presentation of somatosensory target stimulus. Only the TMS on S1 at 20-50 ms post-stimulus caused the illusory disappearance of presented stimulus (extinction). However, the TMS on S2 at 80-120 ms induced the increase of error rate in the intensity judgment task without causing the extinction, suggesting the differential (possibly hierarchical) function of S1 and S2 in somatosensory cognition.

Generation of I-waves in the human: Spinal recordings 1126

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Experimental studies have shown that in response to a single electrical stimulus to the motor cortex, an electrode placed in the medullary pyramid or on the dorsolateral surface of the cervical spinal cord records a series of high frequency waves. The earliest wave that persisted after cortical depression and after cortical ablation was thought to be generated from the direct activation of the fast pyramidal tract neuron axons and was termed “D”. The later waves that required intact gray matter, were thought originate from indirect trans-synaptic activation of pyramidal tract neurons and were termed “I” waves. Recordings from individual pyramidal tract axons showed that a given axon may give both a D and a subsequent I wave discharge.

Recently, the direct recording of epidural acitivity in conscious humans has shown that monophasic magnetic stimulation with a figure-of-eight coil, inducing posterior-anterior current in the brain, evokes a high-frequency (approximately 700 Hz) repetitive discharge of corticospinal axons. Thus, transcranial magnetic stimulation, similar to the direct stimulation of the exposed motor cortex in animals, may produce I-waves.

The direct recording of corticospinal volleys in conscious humans demonstrates that changes in cortical excitability are paralleled by changes in the I-waves. An increase in cortical excitability produced by voluntary contraction determines an increase in the size and number of I-waves. Repetitive transcranial magnetic stimulation at 5 Hz with suprathreshold stimuli also leads to an increase in excitability of I wave mechanisms. Low doses of ketamine can increase motor cortex excitability to transcranial magnetic stimu-

lation. Because ketamine produces a differential modulation of glutamatergic activity, decreasing NMDA neurotransmission and activating non-NMDA transmission, the observed increase in motor cortex excitability suggests that transcranial magnetic stimulation evokes I-waves through the activation of non-NMDA glutamatergic connections within the motor cortex. When the excitability of the motor cortex is decreased the I-waves are inhibited. A decrease in the level of cortical excitability can be produced by a pharmacological enhancement of inhibitory GABAergic activity through the administration of a benzodiazepine such as lorazepam, which determines a pronounced suppression of later I-waves. I-waves inhibition may also be determined by two different protocols of paired cortical stimulation. One is a short latency, low threshold inhibition that is believed to involve the GABAA receptor. The other is a high threshold, long latency inhibition of the I-waves. Moreover, afferent inputs may suppress later I-waves. Since this latter inhibitory effect is reduced or abolished by intravenous injection of the muscarinic antagonist scopolamine, it can be hypothesised that it depends on cholinergic connections.

The sensitivity of the I-waves to the level of cortical excitability supports the hypothesis that they are generated within the cortical networks and that they are presynaptic in origin. The changes in the output of the motor cortex to transcranial magnetic stimulation produced by different pharmacological manipulations, suggest that I-waves are generated by glutamatergic circuits and modulated by GABAergic and cholinergic connections.

1127 Surround Inhibition

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Center-surround organization of the sensory systems of the central nervous system is well accepted and acts to sharpen borders of sensory stimuli. Similar organization should exist in the motor system and could help to make more precise movement. When a specific movement is executed, other possible movements should be suppressed. This concept has been suggested theoretically, some aspects of brain anatomy and physiology seem appropriate, and the basal ganglia, in particular, could be organized to facilitate center-surround mechanisms. There is evidence now for surround inhibition in human movement. Leocani et al. evaluated corticospinal excitability of both hemispheres during a choice auditory reaction time task for extension of either left or right thumb. Transcranial magnetic stimulation (TMS) induced motor evoked potentials (MEPs) simultaneously in the extensor pollicis brevis muscles bilaterally. MEP amplitudes on the side of movement increased progressively in the 80-120 ms before EMG onset, while the resting side showed inhibition. This has been extended by Sohn et al. who showed inhibition of the MEP in the abductor digiti minimi with movement of the flexor digitorum sublimis in the same hand. Liepert et al. showed an increase in intracortical inhibition in a hand muscle after practicing a task where that muscle was to be kept relaxed. Failure of surround inhibition might characterize dystonia, and there is some evidence for this. Bütefisch et al. have shown that task-dependent modulation of inhibition within the motor cortex is impaired in dystonia using an experimental design similar

to that of Liepert et al. Sohn et al. and Molloy et al. have shown defective inhibition in dystonia of the surround muscle with a finger movement.

TMS and Single Unit Recordings in the Visual Cortex of Cat 1128

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Transcranial magnetic stimulation is a safe and well established method for modulating neuronal electric activity. Single pulses are usually applied to test the functional aspects of cortical areas, like excitability, topography, connectivity and specificity, while repetitive (rTMS) application is increasingly used to modify long term cortical excitability as a therapeutic tool. Little, however, is known about the effects of TMS at the cellular level. One basic question is the kind of neuronal processes excited by this method. Calculations based on strength and orientation of the induced electric field indicate that neuronal processes have to travel for some distance along the major axis of the electric gradient to be sufficiently depolarized. Thus, in a cortical area oriented almost parallel to the plane of the inducing coil current, the most likely structures to be excited will be horizontally running axons (1). Results obtained with stimulation of motor cortex with different electric field orientations support this view (3,4,5). To get further insight in the kind of electrical activity induced by TMS in a cortical network, we studied the effect of single TMS pulses of different strength on spontaneous and visually evoked single unit activity in primary visual cortex of the anesthetized and paralyzed cat. Appropriate amplifier settings allowed for extracellular spike detection starting 5 ms after artifact onset. Depending on strength (percent of maximal power), a single pulse could evoke 2 sequences of excitation and inhibition of spontaneous or visually elevated activity. Early, most likely direct excitation (5-20 ms) was followed by an early inhibition lasting up to 150 ms. A second facilitation occurred between 100-500 ms which was followed by another inhibitory episode lasting several seconds (1-4 s). The long-lasting inhibition was found even with moderate TMS intensity (30-50%) while early excitation and inhibition strongly grew at high stimulus intensities. Application of a single TMS pulse at different times relative to a visual response evoked by a moving bar stimulus yielded similar results: inhibition of visual activity when the interval between TMS and visual response was larger than 500 ms and prevailing facilitation for shorter intervals. Facilitation of visual responses was larger than the algebraic sum of visually and magnetically evoked activity, indication a subthreshold depolarization most likely by intraareal lateral inputs. The finding of excitation between 100-500 ms but inhibition following thereafter may give one hint for the dampening effect of slow frequency rTMS (< 1Hz) versus facilitation observed with high frequency rTMS (> 1 Hz). A low stimulation frequency will allow for the development of late inhibition with each stimulus while it might be overcome by the excitatory volley with short inter-stimulus intervals (<500 ms).

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1129 Neurophysiological Effects of Magnetic Seizure Therapy (MST) in Monkeys and Humans

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Convulsive therapy remains an important treatment for severely depressed patients. A better understanding of the therapeutic action of seizures should guide refinements in the technique and may improve its risk/benefit ratio. Seizures differ markedly in their therapeutic benefit and side effect profile. Recent research has shed light on the relationships among the electrical stimulus used to elicit the seizure, the characteristics of the induced seizure, and the clinical outcome (in terms of both therapeutic and ancillary effects). Electrical current density and seizure initiation in prefrontal cortex are associated with the most effective forms of treatment. This suggests that strategies to focus current and seizure initiation in prefrontal cortex may enhance efficacy. Likewise, limiting current and seizure spread in medial temporal lobes might be expected to reduce side effects. The application of an electrical stimulus across the scalp, as with conventional ECT, results in significant shunting of current through the scalp and skull and offers little control over current spread. Since magnetic fields pass through tissue without impedance, seizure induction using a rapidly alternating magnetic field offers the promise of more precise control over induced electrical current and seizure initiation. Magnetic seizure therapy (MST) involves the induction of a seizure under general anesthesia using transcranial magnetic stimulation. We recently demonstrated the feasibility of MST in nonhuman primates and in patients with major depression. We now present data on the physiological characteristics of electrically and magnetically induced seizures in patients with depression and in nonhuman primates.

MST was performed with a custom modified MAGSTIM repetitive magnetic stimulator with 16 booster modules. ECT was performed using a MECTA SPECTRUM device. Anesthesia for both ECT and MST in patients and animals consisted of methohexital (0.75-1.0 mg/kg iv) and succinylcholine (0.75-1 mg/kg iv). Patients (n=22 to date) with a major depressive episode received MST under general anesthesia. Two channels of scalp EEG (bilateral frontomastoid) were recorded at each treatment, and 18-lead EEG was recorded at the 2nd and penultimate treatments. Rhesus monkeys (3-11kg, n=19 to date) received 1-6 week courses of daily MST, electroconvulsive shock (ECS) or sham (anesthesia alone). Scalp and intracerebral EEG (30 recording sites in frontal and temporal cortex) were recorded.

Successful seizure induction was accomplished with MST in all subjects. Focal seizures were observed in some subjects. MST-induced seizures were shorter than ECT/ECS-induced seizures ($p<0.004$). Quantitative EEG analysis revealed that ictal EEG expression was less robust with MST than with ECT/ECS across most parameters examined (p's<0.0005). MST demonstrated less postictal suppression than ECT ($p<0.02$). Topographical patterns of seizure spread measured from scalp leads and intracerebral electro-

des differed between the modalities. MST-induced seizures demonstrated less robust spread to hippocampus and ventral structures than ECS.

Magnetically and electrically induced seizures differ across several physiological measures. The clinical significance of these differences in terms of side effects and efficacy of convulsive therapy is yet to be determined. MST in the nonhuman primate offers a model for the systematic exploration of dosage factors relevant for subsequent clinical trials with MST.

Long term effects by rTMS in humans and monkeys 1130

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rCBF changes during rTMS over M1 in humans We studied effects of 1Hz repetitive transcranial magnetic stimulation (rTMS) over the left primary motor cortex (M1) on regional cerebral blood flow (rCBF) using single-photon emission computed tomography (SPECT). 1Hz rTMS at an intensity of 1.1x active motor threshold (never elicited any movements) evoked increase of rCBF in the contralateral (right) cerebellar hemisphere. Reduction of rCBF was observed in the contralateral M1 and some other areas. Increase of rCBF in the contralateral cerebellum must reflect facilitatory connection between the motor cortex and contralateral cerebellum. Reduced rCBF in the contralateral M1 may be produced by transcallosal inhibitory effect of the left motor cortical activation. rTMS over the right DLPFC in humans rTMS over the DLPFC has been reported to have an antidepressant effect. To clarify mechanisms of slow rTMS over the right DLPFC, we measured rCBF during sham, during real rTMS and after stimulation using repeated ^{15}O-labeled H_2O PET scanning. Slow rTMS over the right DLPFC produced significant rCBF increase in the ipsilateral anterior cingulate cortex (ACC) during stimulation, and the lasting activation was noted in the ventral striatum. These data indicate that slow rTMS is able to produce rCBF changes in the distant brain areas including paralimbic system and frontal cortex. We conclude that such rCBF changes may explain antidepressant effects of rTMS.

Coil for monkeys To stimulate a certain brain, we should use a coil appropriate for the target brain and curvature of the skull. We made a coil which fits the curvature of the skull of monkeys. We measured induce currents in a model of the monkey's skull evoked with this coil and a figure of eight coil for humans. Induced currents were twice or more in stimulation using our coil than that with a usual coil.

rTMS over M1 in monkeys In monkeys, using a PET method, we studied regional glucose metabolism during and after rTMS over M1 with the above coil for monkeys. We gave 20 trains of 5Hz, 100 stimuli in 19 minutes (total 2000 stimuli). The intensity was set to induce the same current density as that induced by a figure eight coil in the human brain model at an intensity of the motor threshold for active muscles. The currents evoked in the monkey brain are supposed to activate the motor cortex but not to induce any movements. At the sites under the coil (M1) and some areas tightly connected with M1, glucose metabolism increased just after rTMS. In some of them, the increase continued to be present even a few days after rTMS. We have shown that rTMS elicits an effect

lasting longer than a few days, which supports a clinical therapeutic beneficial effect of rTMS. Our study of endogenous dopamine release by rTMS over M1 using raclopride revealed that rTMS over M1 released endogenous dopamine at the basal ganglia in the monkey brain. This may partly explain clinical effects of rTMS.

1131 Functional inhibition in the surround of experimental focal cortical dysplasias

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Abstract has not been submitted.

1132 Waves: Their Phylogeny And Cortical Network Origin

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Electrical or transcranial magnetic stimulation of mammalian motor cortex can elicit a synchronous direct (D) response by corticospinal neurons, followed by a synaptically mediated, indirect (I) complex. The pyramidal tract (PT) or corticospinal tract (CT) response of primates is composed of a single D wave and several discrete I waves, with intervals of 1.4 – 2.0 ms (500 – 700 Hz). Synchrony of fast CT fiber discharges in each I wave is extraordinary in monkeys and best in humans, even at conduction distances of 300 mm. Synchrony is reduced in cats and minimal in rats, where the I complex is a single broad wave.

The origin of I waves has been debated for decades. Any proposed mechanism must account for the observed conservation of periodicity despite changes in intensity or site of stimulation. The leading hypotheses for the generation of multiple synchronous I waves include: (1) an intrinsic neuronal membrane periodicity; (2) prolonged transmitter action from a transient input volley; and (3) repetitive synaptic action by an excitatory interneuronal network.

The first two hypotheses can account for multiple discharges by single neurons, but do not account for synchrony between neurons or the conservation of I wave periodicity. Depolarizing current injection in CT neurons over a range of intensities fails to reveal any evidence of a *preferred* interspike interval equal to the I interval, or its first harmonic. Prolonged transmitter action occurs at many mammalian synapses, resulting in high frequency firing, but the frequency typically is *gradable* as a function of the input intensity.

The excitatory network hypothesis rests first on the demonstration of connectivity between superficial and deep layer neocortical neurons (Lorente de No). Physiological

evidence includes: (a) microstimulation, where superficial motor cortical excitation preferentially excites late I waves, but deep laminar stimulation recruits the monosynaptic, I 1, excitation; and (b) pial cooling selectively abolishes late I waves without reducing the amplitude, or increasing the latency of the I 1 wave. These data favor the existence of a centripetally conducting excitatory chain, each I wave period reflecting the delay for EPSP initiation and the slow rise to firing level that is recorded intracellularly. Intracellular recordings from undamaged, fast CT neurons show cells discharging once for an EPSP, in part due to the destruction of an individual EPSP by an action potential. Multiple I wave discharges by an individual cell would therefore require repetitive synaptic activation. While IPSPs terminate the I wave complex, they lack I wave periodicity, even when the membrane potential is reduced.

An excitatory network hypothesis survives as the mechanism accounting for the single cell and population recording data. Differences in latency of the earliest I wave and number of later I waves may reflect the ill understood details of synaptic efficacies of the diverse inputs to the motor cortical laminae. The phylogenetic trend in the synchrony and periodicity implies that care is needed in applying conclusions from studies of lower mammals to the origin of human I waves.

Functional Connectivity of the Premotor and Motor Cortices 1133

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Previous work from our laboratory used a paired pulse design to show that it is possible to detect short latency suppression from premotor areas onto the hand area of the primary motor cortex (Civardi et al, 2001). Later we found that 1 Hz rTMS of the same area could induce lasting effects on the excitability of MEPs (Gerschlager et al, 2001) and the time course of SICI/ICF (Munchau et al, 2002) in the motor hand area at intensities that were insufficient to evoked any effect when applied to the primary motor cortex alone. Our most recent work (Rizzo et al, 2003) shows that it is possible to reverse the effects on motor cortex by using 5 Hz stimulation of premotor cortex rather than 1 Hz. Effectively we can now determine the sign as well as the magnitude and time course of the after effects from premotor stimulation. Behavioural effects on reaction times and movement times are negligible yet PET and fMRI experiment show that this may be because there is compensation for the physiological changes by additional activation in unstimulated parts of the brain. The interaction between premotor and motor cortex may be a model that can be used to investigate other cerebral circuits with TMS.

1134 Interactions between different inhibitory systems in the motor cortex

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Cortical activity depends on the balance between excitatory and inhibitory influences. Several different excitatory and inhibitory systems in the human motor cortex can be tested by transcranial magnetic stimulation (TMS) in conditioning-test paradigm. These include short interval intracortical inhibition (SICI), long interval intracortical inhibition (LICI) and intracortical facilitation (ICF). The motor cortex can also be inhibited by stimulation of the contralateral motor cortex (interhemispheric inhibition, IHI) and by peripheral nerve stimulation at short (short latency afferent inhibition, SAI) and long latencies (long latency afferent inhibition, LAI). Abnormalities of these excitatory and inhibitory measures have been reported in many neurological and psychiatric disorders and in settings of cortical plasticity.

While considerable information is known about these different inhibitory and facilitatory phenomena individually, how they are related to each other and how they interact remain poorly understood. One way to investigate whether experimental phenomena share common mechanism of action is to assess whether their profiles of response are similar or different under conditions of controlled perturbation. We used a method of combining stimulating pulses at short intervals to test interactions of different systems in the human motor cortex. We examined the relationship between short interval intracortical inhibition (SICI) and long interval intracortical inhibition (LICI). We found that different circuits mediate SICI and LICI, and LICI inhibits SICI. The findings support the hypothesis that SICI is mediated by $GABA_A$ and LICI is mediated by $GABA_B$ receptors. In the second study we examined how interhemispheric inhibition (IHI) interacts with SICI and LICI. We found that IHI inhibits SICI, similar to LICI. Both IHI and LICI decreased with higher test stimulus intensities and they may be mediated by a similar neuronal population. We proposed that both LICI and IHI may be mediated by $GABA_B$ receptors and their interaction may be explained by auto-inhibition through $GABA_B$ presynaptic autoreceptors. In the third study we examined how long latency afferent inhibition (cortical inhibition induced by median nerve stimulation (MNSI) at interstimulus interval of 200 ms) interacts with SICI and LICI. We found that MNSI is mediated by circuits distinct from those mediating SICI and LICI. MNSI appear to inhibit LICI while SICI was unchanged in the presence of MNSI. These studies allow us to develop models of interactions between different inhibitory systems that can be tested in future experiments. It also allows better interpretation of abnormal findings with neurological and psychiatric disorder. Testing the interaction of these mechanisms may also be developed into a new way of studying the pathophysiology of neurological and psychiatric disorders in the future and may have diagnostic or prognostic implications.

Paired-pulse TMS: The dimension of stimulus intensity 1135

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Paired transcranial magnetic stimulation (TMS) has greatly advanced our understanding of the mechanisms, which control excitability in human motor cortex. While it is clear that paired-pulse (PP) excitability depends on the exact inter-stimulus interval (ISI) between the first (S1) and second stimulus (S2), relatively little is known about the effects of the intensities of S1 and S2. We proposed that PP measures bear some limitations because usually fixed intensities for the first and second pulse are used, where the conditioning pulse (sub-threshold) was usually already optimal for producing this form of a short-lasting inhibition.

In order to test in greater detail the effects of S1 and S2 intensity on the interaction between S1 and S2 at short ISI less than or equal to 5 ms, we perform several experiments, using four different ISI. Furthermore, at an ISI of 1.5 ms we extend experiment measuring S1-S2 interaction with target muscle in rest as well as under voluntary isometric contraction.

The novel TMS protocol consisted of 81 conditions with variable intensities of S1 and S2 (each given at 9 equidistant intensity steps from 60 to 140% of resting/active motor threshold – RMT/AMT; 5 trials for each condition). The ISI between S1 and S2 was set to 1.5 ms. For comparison, single test stimuli were also given at the 9 different intensities. The interaction between S1 and S2 was calculated as the ratio of MEP amplitudes produced by PP TMS (MEP S1+S2) over the arithmetic sum of the MEPs produced by the single pulses (MEPS1+MEPS2).

The interaction between S1 and S2 was further tested by plotting the function MEPS1+S2 - (MEPS1 + MEPS2) against time. To this end, the individual D-wave latency was determined by anodal TES. In each subject, MEPS1+S2 - (MEPS1 + MEPS2) was then related to the individual D-wave latency of S2, which was assigned a time of zero. Finally, curves were averaged across subjects for each of the 81 conditions of S1 and S2. This approach allows the precise determination of the time course of the interactions between S1 and S2 relative to the D-wave. The onset of interaction was determined as the first consistent deviation of the MEPS1+S2 - (MEPS1 + MEPS2) function away from zero. In order to eliminate the requirement for temporal summation at the neuronal elements along the motor pathway to reach action potential threshold, this analysis is possible to apply in the active muscle only, and only at interaction onset. The correctness of this analysis for a broader sample of neurones, were confirmed with single motor unit recordings.

SICI onset during ADM contraction was at the I3-wave latency of S2, whereas SICF started with the I2- or I1-wave latency of S2. Findings suggest that SICI is mediated through a low-threshold GABA-A receptor dependent inhibitory pathway and summation of IPSP from S1 and EPSP from S2 at the cortico-spinal neurone. In contrast, SICF originates through non-synaptic facilitation at the initial axon segment of interneurones along a high-threshold excitatory pathway.

1136 Paired pulse TMS: Different mechanisms for intracortical inhibition induced by paired pulse TMS at different intervals

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Paired pulse magnetic stimulation (Kujirai et al. 1993) has been widely used to study intracortical inhibition of the motor cortex. Inhibition at interstimulus intervals (ISIs) of 1 ~ 5 ms is ascribed to a GABAergic inhibitory system in the motor cortex. However, different mechanisms may contribute to the inhibition at each interval. In order to confirm their concept and clarify whether inhibition at all these intervals is produced by a single mechanism or not, we compared the effects of the paired-pulse stimulation at ISIs of 1, 2 and 3 ~ 5 ms. We evaluated how intracortical inhibition affected the I3-wave, I1-wave, magnetic D-wave and anodal D-wave components of electromyographic (EMG) responses using previously reported methods (Werhahn et al. 1994, Sakai et al. 1997).

The data suggest that three separate effects occur within these ISIs. At ISIs of 3 ~ 5 ms, inhibition was evoked only in responses to I3-waves, whereas no inhibition was elicited in responses to I1-waves or magnetic D-waves. In contrast, at an ISI of 1 ms, responses to I3-waves and I1-waves were moderately suppressed. Moreover, even magnetic D-waves were slightly suppressed, whereas anodal D-waves were unaffected. At an ISI of 2 ms none of the descending volleys were inhibited.

We propose that we should use ISIs of 3 ~ 5 ms for estimating function of the GABAergic inhibitory system of the motor cortex by paired pulse TMS. Our results support the idea by Fisher et al (2002) that the mechanism responsible for the inhibition at an ISI of 1 ms is not the same as that responsible for suppression at ISIs of 3 ~ 5 ms (GABAergic inhibitory circuits in the motor cortex). At an ISI of 2 ms, we suggest that inhibitory influence evoked by the first stimulus (S1) should be collided with or occluded by the second stimulus (S2), which leads to the lack of inhibition when the subjects make a voluntary contraction of the target muscle.

To consider the above findings and the fact that we should use subthreshold for active muscles as the conditioning stimulus to ascertain the observed effect to be a cortical event, we propose the following methods to study the intracortical inhibition of the motor cortex. The intensity of the conditioning stimulus should be 5 % of the maximum output of the stimulator below the active motor threshold or less, and the test stimulus should be set to elicit mainly I3-waves. In practice, antero-posteriorly directed currents in the brain are give as the test stimulus when subjects make a voluntary contraction(conrrol response size 0.3-0.5 mV), or postero-anteriorly directed currents are given to elicit a control response 0.5-1 mV in size when they relax the target muscle. Interstimulus intervals should be 3-5 ms.

The organisation and reorganisation of human swallowing motor cortex 1137

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Swallowing is a complex sensorimotor activity regulated by interactions between centres in the cortex and brainstem. Following cerebral damage, swallowing problems can occur leading to serious morbidity, in particular malnutrition and pulmonary aspiration. Despite this, swallowing can recover within weeks to a safe level in many patients. This propensity for recovery is likely to relate to how swallowing motor cortex is organised and then reorganised after cerebral injury. A better understanding of these processes may therefore help in developing therapeutic interventions that can drive plasticity and so encourage the recovery process. In this presentation, I will examine present knowledge about the cortical control of swallowing in man utilising "functional imaging" modalities, including TMS, and explore what aspects of its organisation are important for compensating for recovery after damage. In addition I will describe how approaches with TMS may be useful in studying interventions that may drive cortical plasticity and speed up the recovery process. Swallowing may turn out to be an excellent model for study plasticity, providing insight into the physiology and pathophysiology of the human motor cortex.

Inhibitory control of acquired motor programmes in the human brain 1138

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An important basis of skilled human behavior is the appropriate retrieval of acquired and memorized motor programmes ('motor memory traces'). Appropriate retrieval is warranted if motor programs are only activated if necessary and are, probably more often, inhibited if required by the context of a given situation. It is unknown how this type of inhibition is accomplished in the brain. We studied context-dependent modulation of motor memory traces in 18 volunteers and 6 patients with focal dystonia. Cortical function was assessed with transcranial magnetic stimulation over the motor cortex (M1) and with task-related analysis of oscillatory EEG activity. An activation (ACT) and inhibition (INH) condition were compared. In both, visual cues were presented at 1/sec. In ACT, subjects had to respond to these cues with individual finger movements as learned in a preceding training session. In INH, subjects had to observe the cues without retrieval of motor responses. During INH, inhibitory control of the motor memory trace was confirmed by significant amplitude reduction of motor evoked potentials (MEPs) compared with baseline. This was accompanied by a significant increase of 11-13 Hz oscillatory activity over the sensorimotor areas during INH. During active retrieval of the motor memory traces, the reverse was true (increased MEP amplitudes, decreased oscillatory 11-13 Hz activity). In a small sample of dystonic patients (n=6), the increase

of 11-13 Hz oscillatory activity during INH was consistently absent. The present data demonstrate cortical correlates of appropriate, context-dependent inhibition of motor memory traces. We propose that focal increases of oscillatory activity are instrumental for inhibitory control at the cortical level. This concept is supported by the preliminary observations in dystonic patients who are known to have deficits of inhibitory motor control and in whom these context-dependent focal increases of oscillatory activity were absent.

1139 Modulation of Premotor-Motor Interaction by 1 Hz Subthreshold rTMS in Healthy Subjects and *De Novo* Patients with Parkinson's Disease

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Background: The dorsolateral premotor cortex (PMC) modulates motor cortex excitability. We explored the effects of 1 Hz "inhibitory" rTMS over the PMC in de novo patients with Parkinsons's disease (PD).

Methods: Ten PD patients (mean age 58.3 years) and 7 age- and sex-matched healthy controls were studied. All subjects received 1 Hz rTMS (intensity 80% active motor threshold (AMT), duration 20 min) over the PMC (3 cm anterior to the motor cortex hand area). The left was stimulated in healthy subjects and the PMC contralateral to the clinically more affected side in patients. Before and after rTMS, we determined the AMT, the MEP size and intracortical inhibition/facilitation (ICI/ICF) using the Kujirai paired pulse paradigm (conditioning pulses: 80% AMT; interstimulus intervals (ISIs): 2-10 and 15 ms).

Results: Compared with healthy subjects ICI was significantly reduced at an ISI of 5 ms and ICF increased at 8 ms in PD patients. In healthy subjects rTMS produced a significant decrease of ICI at 5 ms and significant increase of IFC at 8 ms. In contrast, reduced baseline ICI at 5 ms was significantly increased in PD patients.

At baseline and following rTMS there was no difference of AMT and MEP size between the groups.

Conclusion: In PD patients motor cortex hyperexcitability can be reduced by 1 Hz PMC rTMS. In contrast, the same rTMS intervention increases motor cortex excitability in healthy subjects. This implies that dopamine deficiency changes the functional interaction between PMC and motor cortex.

Mapping motor cortex projections to single motor units in humans with transcranial magnetic stimulation 1140

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Abstract has not been submitted.

Pharmacology and TMS 1141

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Single application of CNS active drugs has greatly enhanced our understanding of the mechanisms underlying the various TMS measures of motor excitability.

(1) Motor threshold (MT) is the minimum TMS intensity to elicit a small motor evoked potential (MEP) in a target muscle. Blockers of voltage-gated sodium channels (e.g. carbamazepine) increase MT, while drugs acting on neurotransmission do not affect MT. This suggests that MT depends largely axon excitability regulated by voltage-gated sodium channels.

(2) The MEP intensity curve typically displays a sigmoid increase of MEP size with TMS intensity. Slope and plateau of the MEP intensity curve depend much stronger than MT on synaptic excitability. Adrenergic drugs (amphetamine, methylphenidate) and serotonin enhance the MEP intensity curve, while GABAA receptor agonists (e.g. benzodiazepines), anti-adrenergic drugs (guanfacine), and also blockers of voltage-gated sodium channels depress it.

(3) The cortical silent period (CSP) refers to a TMS-induced interruption of voluntary activity in a target muscle. The available data suggest that this long-lasting inhibition (CSP in hand muscles lasts up to 200-300 ms) is mediated by GABAB receptors.

(4) Paired-pulse TMS tests interactions between the effects of two pulses. If the first pulse is < MT and the second pulse is > MT, the interaction is inhibitory at short inter-stimulus intervals of 1-5 ms. This short-interval intracortical inhibition (SICI) is enhanced by GABAA receptor agonists, dopamine receptor agonists, anti-adrenergic drugs and glutamate receptor antagonists but depressed by dopamine receptor antagonists and adrenergic drugs. Blockers of voltage-gated sodium channels do not affect SICI. This suggests that SICI may reflect a net inhibition consisting of strong GABAA receptor mediated inhibition and weaker glutamatergic excitation.

(5) At longer inter-stimulus intervals of 7-20 ms, the interaction is facilitatory. This intracortical facilitation (ICF) has a similar pharmacological profile compared to SICI. ICF is suppressed by GABAA receptor agonists and glutamate receptor antagonists. Most likely, ICF is a net facilitation consisting of weak GABAA receptor mediated inhibition and stronger glutamate receptor dependent excitation.

(6) If both pulses are approximately of MT intensity, or if the first pulse is > MT and the second pulse is < MT, the interaction is facilitatory at discrete inter-stimulus intervals of around 1.3, 2.7 and 4.2 ms. This so called I-wave facilitation is suppressed by GABAA receptor agonists, suggesting that it is controlled mainly by inhibition through the GABAA receptor.

(7) MEP inhibition occurs when an electrical stimulus to the median nerve precedes contralateral TMS by 19-25 ms. This short-interval afferent inhibition is suppressed by antagonists of the muscarinic acetylcholine receptor (scopolamine). Therefore, short-interval afferent inhibition contrasts with SICI which is regulated through the GABAA receptor but remains unaffected by scopolamine.

In sum, acute effects of CNS active drugs allow the dissociation between TMS measures of mainly axon versus synaptic motor excitability. In addition, various neurotransmitter systems can be specifically tested. One first example is the distinction between several types of cortical inhibition which are regulated mainly by the GABAA receptor (SICI), the muscarinic acetylcholine receptor (short-interval afferent inhibition), or the GABAB receptor (CSP).

1142 Effective connectivity of the human frontal cortex and its modulation by repetitive transcranial magnetic stimulation.

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Three principal reasons have motivated recent advances in combining transcranial magnetic stimulation (TMS) with brain mapping and its use to study brain-behaviour relationships in health and disease. First, TMS allows the investigator to manipulate neural activity in space and time and, as such, provides a tool for testing the causality of structure-function correlations revealed by functional imaging techniques. Second, TMS combined with a concurrent measurement of neural activity with PET, fMRI or EEG serves as a behaviour-independent assay of cortical excitability and connectivity. Third, the measurement of TMS-induced changes in neural activity in general, and specific neurotransmitter systems in particular, furthers our understanding of the potential treatment effects of brain stimulation and the pathophysiology of certain brain disorders. This talk will illustrate some of the ways in which TMS can be combined with brain imaging to achieve the above goals, focusing in particular on studies of cortical connectivity of the human frontal cortex and its modulation by repetitive TMS.

Paus, T. Combination of Transcranial Magnetic Stimulation with Brain Imaging. In: J. Mazziotta, A. Toga (Eds). Brain Mapping: The Methods. Second Edition Academic Press, pp. 691-705, 2002.

Interleaving fMRI and rTMS 1143

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Transcranial magnetic stimulation (TMS) can be interleaved with rfMRI to visualize regional brain activity in response to direct, non-invasive, TMS stimulation. We would like to think it is a promising tool for studying brain function. This talk will give a brief overview of what we are doing to try to convince ourselves that that is true.

Review of practical problems encountered in interleaving fMRI with rTMS. We will briefly review the practical issues involved in performing interleaved fMRI and rTMS, including safety, the problems with static and dynamic artifacts, our solution for the problem of radiofrequency interference, and suggest methods for minimizing the interaction between the TMS pulse and MR acquisition. *Is BOLD-fMRI a reliable indicator of response to TMS?* In a study of eleven healthy adults, each scanned three times, we assessed both within-subject and between-subject variation of the BOLD response associated with 1 Hz TMS- induced thumb movement (TMS) and compared it with the response associated with a similar, volitionally-induced movement (VOL). BOLD locations and intensities for TMS and VOL were not significantly different, dx=0.9±7.1; dy=2.3±6.0; dz=0.7±4.3 and dI=0.36±1.3, respectively. Coil placement relative to BOLD location varied more than did BOLD location (dx=17.3±9.5, dy=21.8±8.7, dz=11.0±9.0) over repeated studies.

Where are we stimulating - MR-guided rTMS? A major practical difficulty is the accurate positioning of the TMS coil within the MRI scanner for stimulating a particular area of an individual's brain cortical anatomy, especially when there is no overt response associated with that area. We have designed and built a self-contained hardware/software system for MR-guided TMS coil positioning in interleaved TMS/fMRI studies. Phantom calibration studies gave an accuracy for positioning within setups of dx=±1.9 mm, dy=± 1.4 mm, dz=±0.8 mm and a precision for multiple setups of dx=±0.8 mm, dy=± 0.1 mm, dz=±0.1 mm. Preliminary results from the first study to use this system - targeting "motor knob" - have shown 100% success in achieving motor movement with the settings provided by the MR-guidance software.

Can temporal resolution be improved with paired-pulse rTMS? We have combined the TMS paired-pulse technique, a well-characterized physiological tool for testing intracortical inhibition and facilitation, with BOLD-fMRI neuroimaging. A Macintosh G3 laptop controlled the firing of two stimulators , synchronously interleaved with the fMRI acquisition, through a single TMS coil as a list of paired-pulse events with different interstimulus intervals (ISI). The same event list was later used both to remove TMS compromised images and as the paradigm event list for data analysis with SPM to find areas of BOLD activation. A mathematical model made up of a hemodynamic response function multiplied by an exponential recovery function with independent amplitude scaling factors for the different ISI was then used to fit the BOLD response times curves to obtain amplitude scaling factors that might reflect inhibition or facilitation We hope to use this technique both for testing cortical sensitivity in areas other than motor cortex, and for using the BOLD response amplitude dependence on ISI to investigate brain communication at high time resolution.

1144 Methodological considerations for simultaneous TMS and fMRI studies

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Due to the interaction between the static magnetic field of a MRI system and the similarly strong magnetic pulses generated by transcranial magnetic stimulation (TMS), the desirable combination of both methods with complementary strengths in spatial and temporal resolution has been considered as technically impossible. However, in 1997 Bohning et al. reported a first interleaved MRI and TMS experiment. Nevertheless, only few publications addressed technical limitations and practical solutions for combined TMS and fMRI experiments and, in particular, only very few studies have successfully applied both methods simultaneously. In order to clarify the needs for synchronized fMRI/TMS studies, we evaluated the experimental conditions for such studies ranging from the mere presence of a TMS coil to the use of long-lasting TMS pulse trains.

In a first step, we assessed the influence of a TMS coil on the MR image quality which is known to be sensitive to even tiny distortions of the magnetic field homogeneity e.g. caused by the air-filled nasal cavity (susceptibility artefacts). Metallic materials such as amalgam tooth inlays, EEG electrodes or hair clips usually cause much stronger artefacts. It could be demonstrated in phantoms and at a field strength at 2 Tesla that images without serious distortions may be obtained provided adequate precautions are taken, for example, a proper positioning of the section orientation relative to the TMS coil plane. Recent experiments revealed that a reasonable image quality may also be obtained at 3 Tesla.

Secondly, when applying TMS pulses within the magnet but without MRI, one observes an extreme amplification of the noise generated by the discharging TMS coil. The increased loudness is due to an increase of the forces acting on the TMS coil inside the MRI scanner. However, the underlying mechanical vibrations have been controlled by improved materials which make such TMS coils “MRI compatible” and ensure safe conditions for human applications.

In a subsequent step, the effects of TMS pulses on MRI data acquisition were investigated again using phantoms. During imaging the magnetic fields of TMS pulses act like “spoiling gradients” which effectively destroy the excited MRI signal. When applied during slice excitation, the TMS pulses cause long-lasting modulations of the longitudinal magnetization which can be shown to induce pronounced false positive activations in dynamic functional MRI (fMRI) studies of human brain function. In general a complete absence of MRI artefacts requires waiting periods between a TMS pulse and a fMRI acquisition on the order of 100 ms. We therefore established a precise synchronization of TMS pulses and the MRI system which allowed us to perform fMRI experiments using either the classical block design and 4 Hz TMS or event related paradigms with repetitive TMS at 10 Hz.

Finally, we demonstrated that human cortical stimulation by TMS is not affected by the presence of a strong magnetic field as inside a MRI system. For the case of TMS of the

primary motor cortex no differences in individual motor thresholds for eliciting a peripheral muscle twitch were observed inside or outside the scanner. Altogether it may be concluded that synchronized TMS and fMRI studies are both feasible and save for applications to humans.

BOLD MRI interleaved with high-frequency TMS of the motor cortex 1145

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The recent introduction of combined transcranial magnetic stimulation (TMS) and functional magnetic resonance imaging (fMRI) of the human brain allows to directly visualise the effects of brain stimulation with high spatial and temporal resolution. This approach provides a direct measure of physiological signals generated in the brain, rather than indirectly inferring these by behavioural measures. So far, most electrophysiological studies have focused on primary and secondary motor areas and, consequently, early TMS/fMRI studies have been conducted to complement current knowledge of the motor-cortical physiology. Previous fMRI studies have predominantly focused on investigation of low-frequency (1Hz) TMS combined with fMRI. However, high-frequency stimulation protocols have often functioned as a reversible model of acute brain injury and short-term plasticity. Hence, detailed description of the physiological underpinnings of such protocols will permit further insight into the factors generating immediate or lasting effects of short rTMS trains.

Here, synchronised TMS/fMRI (Siemens Vision 2.0T, echo-planar imaging, 2 x 2 mm resolution, 4 mm section thickness) was conducted over the left human primary sensorimotor cortex (M1/S1) to examine changes in cortical hemodynamics resulting from short sub- and suprathreshold stimulation. Repetitive TMS (rTMS) was applied either in an event-related design (1s pulse train at 10Hz) with post-hoc exclusion of images distorted by direct TMS pulse interference, or synchronised with continuous fMRI (10s, 4Hz). In separate experiments, stimulation intensities were set above, below or around the threshold for peripheral muscle activations during rTMS.

Following suprathreshold rTMS, fMRI signal changes in M1/S1 were mostly located in the depth of the central sulcus, rather than in more superficial areas directly underneath the TMS coil. The results indicate a strong contribution of afferent feedback from activated peripheral hand muscles for the induction of fMRI signal changes in the directly stimulated area within M1/S1, and thus largely confirm previous studies using low-frequency stimulation protocols.

Furthermore, in order to detect subtle fMRI responses following rTMS, a region-of-interest analysis was performed in anatomically defined areas such as the supplementary motor areas (SMA), dorsal premotor cortex (PMd), and M1/S1. Increased fMRI signals were found after prolonged suprathreshold rTMS (10s) in bilateral SMA, left PMd, and left M1/S1, whereas decreased signals occurred in contralateral (right) M1/S1. Most interestingly, subthreshold rTMS (10s) revealed fMRI signal increases in SMA and left

PMd but not in left M1/S1. Instead, signal decreases were detected in contralateral (right) M1/S1 in several subjects.

The results point towards effective rTMS-induced modulation of neural activity within anatomically connected brain regions as detected by fMRI. Whereas suprathreshold rTMS over M1/S1 predominantly elicits brain activations in response to processing of afferent feedback (here: peripheral muscle movements), subthreshold rTMS is capable to cause fMRI responses in motor-cortical circuits in the absence of overt motor responses.

1146 Applications of combined TMS-PET studies in clinical and basic research

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Aim: In a series of experiments, we used positron emission tomography (PET) to explore the pattern, time course, and extent of functional changes induced by a single session of repetitive transcranial magnetic stimulation (rTMS).

Methods: Each experiment had a factorial design with the factors “intervention” (real stimulation vs. sham stimulation) and “task” (finger movements vs. rest). 1800 pulses of rTMS was given at 1Hz and 90% of resting motor threshold to the primary motor cortex (M1) or the dorsal premotor cortex (PMd). Lasting effects of rTMS on neuronal activity were assessed by consecutive PET measurements of regional cerebral blood flow (rCBF).

Results: (i.) Real rTMS caused lasting changes in regional neuronal activity at the site of stimulation and in distant brain areas. We found both regional activations and deactivations. Much of distant changes in activity were consistent with activation of anatomical connections from the stimulated areas. (ii.) The after effects on neuronal activity lasted for at least 40 minutes after the end of each session. (iii.) Most of stimulation-induced changes in regional activity were equivalent at rest and during movement. For 1Hz rTMS of the M1, however, there was also a change in the pattern of movement related activation. (iv.) One of the most unexpected findings was the scale of the changes induced by rTMS. In many cortical areas, the magnitude of stimulation-induced activations or deactivations was as large as task related activation seen with movement of the fingers. (v.) Changes in rCBF at the site of stimulation depended on the frontal motor area targeted by rTMS. (vi.) Premotor rTMS resulted in larger after effects in patients with focal dystonia than in healthy controls, indicating that an underlying dysfunction may alter rTMS-induced plasticity.

Conclusion: These results demonstrate that functional brain mapping is a feasible tool to explore how transcranial stimulation interacts with brain function. Combining functional neuroimaging with rTMS opens up new avenues to map representational plasticity in the human brain.

Repetitive Transcranial Magnetic Stimulation in the Treatment of Epilepsy 1147

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In recent years it became evident that repetitive transcranial magnetic stimulation (rTMS) is capable of modulating the excitability of the cortical neuronal network thus inducing neuroplastic changes of the cortex; for the motor cortex it was shown that high-frequency rTMS (> 1Hz) enhances excitability whereas low-frequency rTMS (< 1 Hz) results in reduced excitability. The physiological mechanism underlying this phenomenon is fairly unknown. Based on the results of single case studies it was hypothesized that reduction of cortical hyperexcitability in epilepsy patients could be of therapeutical potential. In a pilot study on 9 patients suffering from drug-resistant epilepsies we have shown that rTMS with 0,3 Hz on five consecutive days reduced seizure frequency by on average 38% over a period of four weeks (Tergau et al., 1999). However, the first placebo-controlled study in 24 patients published so far (Theodore et al., 2002) only showed slight reduction in seizure frequency by 16% failing significance. Currently, we are performing a multicenter controlled trail comparing in a cross-over design rTMS with 0,3 Hz and 1,0 Hz against placebo stimulation. First results of only 9 patients (Tergau et al., 2002) yielded significant improvement by mean reduction in seizure frequency of about 35% and, furthermore, gave evidence that 0,3 Hz rTMS may be superior to 1 Hz rTMS, albeit interindividual variability seems to be high. At the time of the conference, presumably 16-20 patients will have finished the study and detailed results will be presented. At present, it seems obvious that besides e.g. frequency, intensity and focality several other stimulation parameters and factors may influence the efficacy of rTMS treatment in different epilepsy syndromes which will be discussed. More investigation is needed to prove rTMS evolving as non-invasive therapy strategy in medically intractable epilepsies.

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Theodore et al., Neurology, 2002, 59: 560-562

RTMS for the treatment of pain 1148

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Invasive electrical stimulation of the motor cortex (MCS) has been reported to be of therapeutic value in pain control. We were interested whether non-invasive repetitive transcranial magnetic stimulation (rTMS) of the primary motor cortex might also act

beneficially. We enrolled twelve patients with therapy-resistant chronic pain syndromes (mean age 51.3 ± 12.6, 6 males) in a pilot study. They were treated with rTMS of the corresponding motor cortex area for 20 min (20 Hz, 20 x 2 s trains, intensity 80% of motor threshold) and sham stimulation (sequence-controlled cross-over design). Some of the patients had an analgesic effect, but for the whole group, the difference between active and sham stimulation did not reach a level of significance (active rTMS: mean VAS reduction -4.0% ± 15.6; sham rTMS: -2.3% ± 8.8). In particular one patient suffering from intractable pain due to a cervical myelopathy reported a 25% pain reduction immediately after treatment which persisted for 9 days. Further studies using different rTMS stimulation parameters (duration and frequency of rTMS) or stimulation sites (e.g. anterior cingulate gyrus) are encouraged.

1149 Right prefrontal TMS vs sham TMS in mania: A controlled follow-up study

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ECT is effective in mania as well as depression. Since TMS may have ECT-like properties, we performed a study (Grisaru N, Chudakov B, Yaroslavsky Y, Belmaker RH (1998) Am J Psychiatry 155:1608-1610) of TMS in mania. A Cadwell High Speed Magnetic Stimulator with a 9 cm diameter circular coil was used. Each patient was assessed for motor magnetic threshold before the first treatment and 80% of individual patient motor threshold was then administered for all treatment days. Of the sixteen completers, 12 were manic nonpsychotic, 4 were manic psychotic. Patients were given 10 daily consecutive sessions, 20 trains per session. Frequency was 20 Hz for 2 seconds per train; intertrain interval was 1 min. Each of the participants was given the stimuli over the right prefrontal cortex or the left prefrontal cortex, as randomized. Mean patient motor threshold was 67% for the left treatment group (range 50% to 80%) and 72% for the right treatment group (range 55% to 85%). For Mania Scale two-way ANOVA (with repeated measures) with covariance for baseline showed a highly significant effect of time ($p < .0001$) and significant interaction of time and side of TMS ($p = .012$). Post-hoc-sheffe test showed a significant effect of side of TMS at day 7 ($p = .03$) and 14 ($p < .001$). Results were similar for total BPRS, CGI and BPRS mania factor. These results suggest that TMS stimulation in mania of the right prefrontal cortex has therapeutic effects. However, it is also possible from these results that left prefrontal TMS worsens mania. Therefore we studied TMS right prefrontal stimulation vs sham TMS in mania in a design identical to that above.

Methods: Twenty-five patients entered and 19 completed right TMS vs. sham right TMS. Results: Right TMS was no more effective than sham TMS. Conclusions: It is possible that the previous results were due to an effect of left TMS to worsen mania. Alternatively, it is noted that the present patient group had much more psychosis than the previous study of TMS in mania, and depression studies have reported that psychosis is a poor prognostic sign for TMS response.

The neurobiological basis of therapeutic use of rTMS in psychiatric disorders

1150

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Potential therapeutic properties of rTMS have been suggested in several psychiatric disorders such as depression and schizophrenia. rTMS has the potential to either directly or trans-synaptically modulate neuronal circuits thought to be dysfunctional in these psychiatric disorders. To use rTMS optimally, knowledge concerning the putative neurobiological changes underlying the observed clinical effects is indispensable. Preclinical studies in suitable animal models and basic studies at the cellular and molecular level are necessary to understand how the induced intracerebral current density is regulated and which regulatory elements might serve as potential treatment targets. There is compelling evidence that rTMS causes changes in neuronal circuits as reflected by behavioural changes, and decreases in the activity of the hypothalamic-pituitary-adrenocortical system. These alterations are reminiscent of those accompanying antidepressant drugs and suggest regional changes in neurotransmitter/neuromodulator release, signaling pathways and in gene transcription. Indeed, specific changes in the dynamic release patterns of biogenic amines, amino acids and the neuropeptide vasopressin in response to rTMS could be demonstrated in our laboratory by use of the microdialysis technique. Our data provide evidence that acute rTMS of frontal brain regions has a modulatory effect on both the mesolimbic and the mesostriatal dopaminergic systems, i.e. the dorsal hippocampus, the nucleus accumbens and the striatum. Given the beneficial effects of rTMS in the treatment of affective disorders, the mesolimbic dopaminergic system is of particular interest as it comprises the major components of the neural circuitry of reward and incentive motivation. In addition, the increase in dopaminergic neurotransmission may contribute to the beneficial effects of rTMS in the treatment of Parkinson´s disease. With respect to the design of clinical trials, the identification of patients with a putative deficit in dopaminergic neurotransmission related to psychopathology might lead to a better antidepressant efficacy of rTMS beyond the only moderate therapeutic effects reported so far.

Exposure to psychotropic drugs or stress regulates the rate of neurogenesis in adult brain, suggesting a possible role for neurogenesis in the pathophysiology and treatment of neurobiological illnesses such as depression. We therefore investigated whether a concomitant rTMS treatment might exert beneficial effects on chronic psychosocial stress-induced decrease in adult hippocampal neuron production and/or survival of the newly generated cells. However, despite an increase in brain-derived neurotrophic factor (BDNF) and in contrast to antidepressant drug treatment, we failed to reveal beneficial effects of rTMS on adult hippocampal neurogenesis.

The studies were supported by the German Federal Research Ministry within the promotional emphasis "Competence Nets in Medicine".

1151 RTMS in Psychiatry, What do we Really Know?

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Repeated daily prefrontal rTMS has shown antidepressant effects in over 20 small sample randomized controlled comparisons, five separate meta-analyses of these studies, and in randomized trials with electroconvulsive therapy. However, the samples sizes of these studies have been relatively small and the TMS stimulus administered may not have been of an adequate dose in terms of the intensity of stimulation or length of treatment in order to demonstrate an optimal antidepressant effect. Recent scientific evidence and pilot data support the fact that the antidepressant response to rTMS is dose-dependent, with respect to intensity of stimulation, and length of treatment. These findings will be reviewed. Additionally, many brain imaging studies .have been performed in patients receiving TMS. These imaging studies provide clues as to potential mechanisms of action, and point the way to future hypothesis-driven applications of TMS in psychiatric disorders.

1152 Repetitive Transcranial Magnetic Stimulation and Electroconvulsive Therapy in Resistant Major Depression. A Comparison Study.

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Background: Studies published over the past few years suggest that TMS may have significant antidepressant actions. In a previous report we compared ECT and rTMS and found ECT superior for psychotic Major Depression (MD), however ECT and rTMS had similar results in non-psychotic MD. We now report on a controlled randomized comparison of ECT and rTMS in patients with non-psychotic MD.

Methods: Forty patients with non-psychotic MD referred for ECT were included. ECT was performed according to established protocols. RTMS was performed over the left dorsolateral prefrontal cortex at 90% motor threshold. Patients were treated with 20 sessions (5 times a week for 4 weeks) of 10 Hz treatments (1200 pulses per treatment-day) at 90% MT. Response to treatment was defined as a decrease of at least 50% in the HRSD score with a final HRSD of less than 10 points, and a final GAF of 60 or more points.

Results: The overall response rate was 58% (23 out of 40 patients responded to treatment). In the ECT group 12 responded and 8 did not; in the rTMS group, 11 responded while 9 did not (χ square .1 NS). Thus, patients responded as well to either ECT of rTMS.

Conclusion: This study adds to the growing literature supporting an antidepressant effect for rTMS. This study is particularly relevant because it suggests that rTMS and ECT reach similar results, in non-psychotic MDD.

RTMS of the Prefrontal Cortex in Major Depression: Mechanisms of Action and Clinical Efficacy 1153

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Transcranial magnetic stimulation (TMS) has become a major research tool in experimental clinical neurophysiology due to its potential to non-invasively and focally stimulate cortical brain regions. Currently, studies are being conducted to investigate whether rTMS-mediated modulation of cortical function may also provide a therapeutic approach in neurological and psychiatric disorders. Preclinical findings have shown that prefrontal rTMS can modulate the function of fronto-limbic circuits, which is reversibly altered in major depression. rTMS has also been found to exert effects on neurotransmitter systems involved in the pathophysiology of major depression, e.g. to stimulate subcortical dopamine release and to act on the hypothalamic pituitary adrenal (HPA) axis, which is dysregulated in depression (1,2). To date numerous open and controlled clinical treatment trials, with widely differing stimulation parameters, have explored the antidepressant potential of rTMS (3). Though conducted with small sample sizes, the majority of controlled trials demonstrated significant antidepressant effects of verum rTMS compared to a sham condition. Effect sizes, however, varied from modest to substantial and patients were rather therapy-resistant in the majority of studies. Moreover, the average treatment duration was about two weeks, which is short compared to other antidepressant interventions. Larger multicenter-trials, which would be mandatory to demonstrate the antidepressant efficacy of rTMS, have not been conducted to date. A putative future application of rTMS may be the treatment of patients who did not tolerate or did not respond to antidepressant pharmacotherapy prior to more invasive means as electroconvulsive therapy and vagus nerve stimulation. Theoretically, rTMS may be also applied early in the course of disease in order to speed up and increase the effects of antidepressant pharmacotherapy. However, this application has not been a focus of clinical trials to date. Efforts should be intensified to further investigate the efficacy of rTMS as antidepressant intervention and to test specific applications in the treatment of depressive episodes.

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1154 Transcranial magnetic stimulation and cognitive Neuroscience

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Abstract has not been submitted.

1155 The role of the frontal eye fields and posterior parietal cortex in visual search

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The respective roles of the parietal cortex and frontal eye fields will be discussed. There is widespread belief that (a) the parietal cortex is important for something that is loosely termed binding and (b) that the frontal eye fields are important only for the oculomotor aspects of visual scene analysis rather than having any role in feature analysis. In our own experiments we have used transcranial magnetic stimulation in human subjects to delineate the conditions under which the parietal cortex is of particular importance in search and find these to be limited to situations in which the subjects are naive, the location of the target is unpredictable and two or more feature variables are involved. Thus a simple binding account does not explain the role of the parietal cortex. Work on the frontal eye fields (FEF) shows that the right FEF is important in search but the left is not and also shows that the critical timing of the FEF effect is between 40-80 msecs, ie earlier than the effects on the parietal cortex. Following from physiological work in non-human primates, it seems that the FEF is involved in feature-related processing when eye movements are not required. We therefore argue that both the widespread beliefs (a) and (b) above require some reassessment.

1156 Phosphenes and visual suppression by occipital TMS

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When applied over the occipital pole, transcranial magnetic stimulation (TMS) disrupts visual perception and induces phosphenes. Both the underlying mechanisms and the brain structures involved are still unclear. We investigated the emergence of phosphenes in relation to perimetric measurements. The coil positions were measured with a stereotactic positioning device and stimulation sites were characterized on the basis of individual retinotopic maps measured with fMRI. Phosphene thresholds were found to lie a factor of 0.59 below the stimulation intensities required to induce visual masking. They covered the segments in the visual field where visual suppression occurred with higher stimulation intensity. Both phosphenes and transient scotomas were found in the lower visual field in the quadrant contralateral to the stimulated hemisphere. They could be

evoked from a large area over the occipital pole. Phosphene contours and texture remained quite stable with different coil positions over one hemisphere and did not change with the retinotopy of the different visual areas on which the coil was focused. They cannot be related exclusively to a certain functionally defined visual area. It is most likely that both the optic radiation close to its termination in the dorsal parts of V1 and back-projecting fibers from V2 and V3 back to V1 generate phosphenes and scotomas.

Language processing and the motor cortex 1157

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The interference with language processing was one of the first „non-motor“ applications of TMS. Since then a number of studies have explored inhibitory and excitatory effects of TMS and rTMS on various language functions. Another topic of research has been the investigation of functional connections between the motor cortex and language related areas. Recent experiments of the Aachen TMS group in normal subjects have explored in detail the changes of excitability of the motor cortex during reading and during non-verbal mouth movements. During reading aloud excitability was increased in the hand motor area of the language-dominant hemisphere. The excitability of the motor cortex of the right leg or the non-dominant hand remained unchanged. In patients suffering from chronic post-stroke aphasia reading aloud significantly increased the excitability of the motor cortex of the left hand. This result supports the hypothesis that the right hemisphere is involved in the recovery from aphasia. TMS is, therefore, in our opinion a valuable tool to explore the specific functional connections between the hand motor cortex and the cortical language network.

Rapid Experience-Dependent Plasticity in the Human Visual Cortex 1158

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Light deprivation results in increased visual cortex excitability measured as decreased phosphene thresholds following transcranial magnetic stimulation (TMS) over the visual cortex and increased fMRI activation to incoming visual input (Boroojerdi et al., 2000). It leads to adaptive changes in perceptual abilities such as better consolidation of spatial memory in animals and in lower visual recognition thresholds in humans. The mechanisms underlying these rapid adaptive processes are incompletely understood. Using a pharmacological approach and TMS in normal volunteers, we found that lorazepam (which enhances GABAA receptor function by acting as a positive allosteric modulator), dextrometorphan (NMDA receptor antagonist), and scopolamine (muscarinic receptor antagonist) blocked rapid plastic changes associated with light deprivation. Light deprivation alone in a drug-naïve session decreased the phosphene threshold by 20% (Boroojerdi et al., 2001). Additionally, we utilized magnetic resonance spectroscopy

(MRS) in these subjects and measured GABA levels in the occipital cortex of intact humans during light deprivation. Light deprivation led to decreased GABA levels in the occipital cortex paralleling the increased visual cortex excitability. These findings identified GABAergic inhibition, NMDA receptor activation, and cholinergic transmission as mechanisms operating in rapid, experience-dependent plasticity in the human visual cortex.

1159 Fluctuations of motor cortex excitability in pain syndromes

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In patients with chronic pain syndromes, the occurrence and maintenance of chronic pain may be linked to the occurrence of reorganisation in the primary sensorimotor cortex, as it is suggested by different studies in patients suffering from phantom pain after limb amputation (Flor et al.1995, Karl et al. 2001). This cortical reorganisation may be based on cortical excitability changes, resulting from a decreased GABAergic and an increased glutamatergic activity. We therefore applied transcranial magnetic stimulation (TMS) under a paired pulses paradigm to assess intracortical inhibition and facilitation in patients with chronic pain syndromes (chronic phantom pain, complex regional pain syndrome type I = CRPS I) in order to detect changes in the activity of intracortical interneuronal (GABAergic and glutamatergic) circuits.

In patients with chronic phantom pain, we found a reduced intracortical inhibition and an increased intracortical facilitation in the hemisphere contralateral to the amputation compared to healthy subjects. These excitability changes did not correlate with phantom pain intensity in limb amputees. Enhancement of intracortical inhibition and reduction of intracortical facilitation by the administration of the NMDA antagonist memantine was not able to significantly reduce phantom limb pain either. In patients with CRPS I, we found a bilateral disinhibition of the motor cortex, whereas intracortical facilitation did not differ between patients and controls. The amount of disinhibition in the hemisphere contralateral, but not in the hemisphere ipsilateral to CRPS I was associated with the pain intensity as assessed by visual analogue scaling (VAS).

We therefore conclude that there are fluctuations of motor cortex excitability in patients with chronic pain syndromes. However, these excitability changes seem not only to be related to the pain intensity, but also to depend on other factors, such as an alteration of the peripheral input after nerve dissection.

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Transcranial direct current stimulation of the visual cortex 1160

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In the past years, significant achievements have been made in characterizing the properties of striate and extrastriate cortices and to describe their feed-forward and feed-back connections using several neurophysiological techniques in man. Transcranial direct current stimulation (tDCS) offers the possibility of inducing acute and persistent neuronal excitability changes using weak currents, probably by shifting neuronal resting potential. The application of an anodal DC stimulus to the visual cortex increases cortical excitability, while a cathodal current results in a reversed effect. In this lecture we summarize results derived from the use of tDCS on visual perception, including contrast as well as motion detection and visuomotor coordination. We will show that visual functions can be transiently altered by tDCS, as has been shown in the motor cortex previously. Quantitiative results will be presented showing that tDCS of V1 modulates the contrast threshold, the amplitude of the N70 component of visual evoked potentials and phosphene thresholds. Data will be shown that tDCS of V5 and of the primary motor cortex affect visuomotor learning and visuomotor coordination. Our results confirm that tDCS elicits a transient, reversible alteration in the excitability not only of the motor but also of the visual cortex, thus presenting a promising tool for neuroplasticity research.

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Exploring Paradoxical Functional Facilitation with rTMS 1161

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Repetitive transcranial magnetic stimulation can modify the excitability of the targeted cortex beyond the duration of the stimulation train itself. Such modulatory effects appear to depend on the stimulation parameters, particularly on the stimulation frequency. Animal studies provide a unique opportunity to characterize these modulatory effects of rTMS more fully. Studies on the effects of rTMS on 2-deoxy-glucose (2DG) uptake in cats illustrate that low-frequency (1 Hz) rTMS results in a suppression of metabolic (and hence neuronal) activity in the targeted brain area that is focal and trans-synaptically affects activity in distant cortical and subcortical structures depending on the strength of anatomical connections.

In humans, the functional impact of such modulatory effects of rTMS can be shown in neurophysiologic, neuroimaging, and behavioral studies. Experiments have generally employed the approach of disrupting activity in the targeted cortical regions to investigate causal relations between focal brain activity and behavior (‘virtual lesion approach”). Another possible application of such modulatory effects of rTMS is to systematically explore the possibility of paradoxical functional facilitations, i.e. the enhancement of behavior by disruption of focal brain activity.

Studies on the effects of parietal rTMS on visual hemispatial attention and of motor cortex rTMS on sequence motor learning provide examples of such applications of rTMS. Disruption of a targeted cortical region by rTMS results in a behavioral improvement (enhanced ipsilateral attention or speeded-up sequence learning) presumably by releasing distant cortical or subcortical structures from inhibition exerted by the directly targeted (and suppressed) brain region. Such application of rTMS provides novel insights into the distributed interactions among brain regions accounting for a given behavior and may lead to novel application of rTMS in neurorehabilation and neuropsychiatric disorders.

1162 Behavioral and physiological correlates of cortical plasticity: Studies with TMS

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Abstract has not been submitted.

1163 Paired stimulation techniques in conjunction with TMS

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Repetitive TMS, and repetitive peripheral nerve stimulation have each been used separately to induce lasting behavioural and neurophysiological effects in the central nervous system. Experiments performed in experimental animals suggest that the enduring effects of stimulating intracortical pathways may be shaped by simultaneous sustained (e.g., by deafferentation) or phasic manipulations of cortical afferent input. We have used pairs of TMS combined with peripheral nerve stimulation, timed to generate near-synchronous events in the primary motor cortex, to induce lasting changes of corticomotor excitability. The plasticity induced by this paired associative stimulation (PAS) protocol is located in the motor cortex, long-lasting, reversible, topographically specific, and dependent on activation of N-methyl-D-aspartate receptors. These distinct physiological properties resemble those of long-term potentiation (LTP) and long-term depression (LTD) as elucidated in animal experiments. One particularly noteworthy feature of PAS-induced plasticity is that the direction of induced changes of cortical excitability depends on the precise timing of the stimulation modalities. Depression of cortical excitability is induced when the events triggered by TMS precede the events elicited by median nerve stimulation in the motor cortex, whereas enhancement is observed when the sequence of events is reversed. This property may correspond to the dependency on spike-timing observed for bidirectional modulation of synaptic efficacy in many brain regions. PAS-induced changes of cortical excitability alter the pattern of regional activation induced by voluntary movements in fMRI studies while leaving basic motor behaviour unchanged. PAS-induced LTP/LTD-like changes of cortical exchanges may be used to study physiological mechanisms underlying motor learning, may reveal insight into disorders in which mal-adaptive plasticity is thought to play a role and finally

may have a potential as alternative therapeutic tools modulating excitability in neurological disorders where cortical excitability is locally abnormal.

RTMS and Learning 1164

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Learning is the process of acquiring memories, responses, behaviors, procedures, or skills. Memories can be explicit (declarative, conscious) or implicit (unconscious, procedural). Declarative memory is highly associated with hippocampal and parahippocampal function, emotional memory with amygdalar systems, and procedural memory with motor systems. Declarative memory is expressed verbally and can be episodic or semantic. Episodic memory is context-specific for events; semantic memory represents facts that are independent of context. Procedural implicit learning represents improvement in task performance that develops and manifests outside the subject's conscious awareness. In addition, information that is normally considered explicit can be acquired under conditions where the subject denies conscious perception.

As with other cognitive functions, TMS effects on learning are more subtle and more difficult to elicit than those on motor or primary sensory systems. They are most likely to be demonstrated near "threshold"-- that is, with tasks that are sufficiently difficult that performance lies halfway between perfect and random even in the absence of stimulation. TMS investigations of explicit learning are also impeded by anatomy: the hippocampal formation and associated structures of the mesial temporal lobe are distant from the scalp surface, making them difficult to activate directly without causing excess discomfort or exceeding safety recommendations in the overlying cortex. However, portions of the frontal and parietal convexities are strongly connected with hippocampal systems, and appear to serve as gateways for TMS effects on episodic memory.

Multiple studies of TMS over dorsolateral prefrontal cortex have demonstrated effects on learning that extend beyond local working memory. Modalities affected include implicit procedural learning, spatial sequence learning, encoding of word pairs, associative memory for pictures and abstract designs, and retrieval from episodic memory. Apparent dissociations have been noted between different mnemonic functions. Interestingly, some of the discordant results parallel those previously noted on neuroimaging studies. TMS has become a useful noninvasive tool for probing human memory function, but careful analysis of precisely "what and where" is being encoded remains crucial to interpretation of the results.

1165 Cortical and psychophysical effects of rTMS in Hebbian learning

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rTMS (repetitive transcranial magnetic stimulation) is widely used to investigate different aspects of cortical excitability and inhibition, which play an important role in controlling plasticity. We demonstrate, using psychophysical testing and parallel functional magnetic resonance imaging (fMRI), that subliminal rTMS applied with a figure-eight coil positioned over the digit representation of left somatosensory cortex (SI) evokes plastic changes on the stimulated, ipsilateral cortical hemisphere. Two rTMS sessions (5 Hz, 25 trains with 50 single pulses, for 10 min) with a rest period of 1 hour between sessions resulted in selective and reversible reorganization of cortical finger areas in SI. As an indirect marker of cortical reorganization we measured tactile spatial two-point discrimination of the right index (IF) and ring finger (d4) in a AFC task before and after rTMS. The left IF served as control. rTMS revealed a significant lowering of thresholds for the right IF and d4, which was reversible within 135 min for the IF and within 90 min for d4. The left IF was not affected by rTMS. A combined assessment of discrimination thresholds and fMRI recordings revealed a rTMS-induced enlargement of SI, which was linearly correlated with the individual gain of discrimination improvement after rTMS. The results indicate that a stimulation protocol resembling those used in LTP studies, applied from outside directly to selected brain regions can induce meaningful cortical reorganizations paralleled by improvement of discrimination thresholds.

1166 Transcranial Magnetic Stimulation and Cognition

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Few years after its introduction, transcranial magnetic stimulation (TMS) significantly changed the way to investigate brain-behavior relations. Because it disrupts the organized cortical activity, TMS can be understood as a virtual lesion technique which interferes transiently, reversibly and safely with the function of cortical networks involved in cognitive processes. This technique has good spatial and temporal resolution and excellent functional resolution too. Among the many tools today available for imaging the brain activity, magnetic stimulation is the only technique that, allowing to interfere with brain function, offers the possibility to investigate the neurophysiological mechanism underlying cognitive task. This peculiarity of TMS permits to study the relationship between focal cortical activity and behaviour, to establish the temporal connection between a particular cortical region activity and a given task, and to map the functional connectivity between brain regions. A disruption of free recall, working memory, and implicit learning have been demonstrated with frontal cortex stimulation, while frontal and right posterior parietal cortices stimulation gives rise to transitory controlateral

visuo-spatial neglect in normal subjects. The next frontier will be to confirm previous observations about the possibility that repetitive transcranial magnetic stimulation (rTMS) improves cognitive processing and understand specific neuronal networks and subsystems involved.

Motor cortex plasticity after hand amputation 1167

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There is a large body of evidence that long-term deafferentation in amputees leads to excitability changes of the contralateral motor cortex (1, 2). To examine, if there is also a shift of stump muscle representation into the deafferented adjacent cortex area, as was shown in animal studies (3), we studied in 10 patients with long-standing amputation at the level of the forearm spatial changes of the motor cortical representation of the biceps brachii muscle (stump muscle). Motor output maps, established by focal transcranial magnetic stimulation of the motor cortex, had the following characteristics: spatial extension, maximal response amplitude and center of gravity (COG) of the spatial distribution of response amplitudes. The extension of the stump muscle motor maps was increased (ratio: 1.5 ± 0.3 versus 1.0 ± 0.3 in control group, $p<0.05$) and the stump muscle motor responses were much larger (ratio: 2,6 ± 0,6 versus 1,0 ± 0,5 in the control group, $p<0.05$). The center of gravity of the stump muscle map was significantly shifted laterally by, on average, 6.0 ± 7.7 mm (range -3.4 to 21 mm, $p<0.05$), either reflecting gross changes of local cortical excitability or structural anatomic reorganization (4). Interestingly, we found that long-term amputation leads to excitability changes also in the ipsilateral motor cortex. Whereas short-term deafferentation due to ischemic nerve block was reported to increase excitability of the ipsilateral motor cortex (5), we found in our patients an enhanced intracortical inhibition, as assessed with the paired pulse paradigm of Kujirai (6).The mean amplitude of conditioned responses in typically inhibitory Interstimulus-Intervals (1-5 ms) was 30% of test response amplitude ± 27 in amputees, and 59% of test response amplitude ± 27 (mean ± SD) in aged-matched healthy subjects. The difference was significant ($p<0.05$), as tested separately for each interval. This could reflect an interaction, possibly transcallosally mediated, between homotopic sites in the motor cortex of both hemispheres. On the other hand, because our patients used no prosthetic devices but exclusively their residual hand, it could be assumed that the enhanced intracortical inhibition is associated with long-term motor training. In line with this hypothesis are a number of animal studies, which demonstrated the role of local GABA circuit control in learning and experience-dependent plasticity (7, 8).

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1168 TMS in neurorehabilitation

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Abstract has not been submitted.

1169 Modulation of use-dependent plasticity by amphetamine

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The organisation of the primary motor cortex is modified by use, a process often referred to as plasticity. Use-dependent plasticity may play a beneficial role in the functional recovery that follows injury to the central nervous system and in motor learning.

D-amphetamine (AMPH) is a drug that exerts its effect through the presynaptic release of the monoamines noradrenaline, dopamine and serotonin and inhibition of their reuptake from the synaptic cleft. It can enhance the beneficial effects of physical therapy after cortical injury in animal models and in stroke patients, possible by alleviation of injury-induced functional depression of structures remote from the injury site (diaschisis). Because AMPH facilitates behaviorally-assessed memory storage through its effect on memory consolidation and may exert a facilitatory effect on long-term potentiation (LTP), it is possible that AMPH enhances plastic changes that may be involved in the recovery after injury to the brain. Motor training leads to encoding kinematic details of the practiced movements in the human motor cortex, a form of training-dependent plasticity that has been identified in healthy volunteers. We hypothesised that AMPH may facilitate this form of motor memory. Healthy human volunteers underwent a training consisting of voluntary thumb movements under the effects of placebo or AMPH in different sessions in a randomized double-blind, counterbalanced design. Previous work in a drug-naïve condition showed that such training causes changes in the direction of thumb movements evoked by transcranial magnetic stimulation (TMS) and in TMS-evoked electromyographic responses. The endpoint measure of the study was the magnitude of training-induced changes in TMS-evoked kinematic and electromyographic responses in the AMPH and in the placebo conditions. Post-hoc analysis of motor performance during training revealed no differences between the two conditions. Motor training resulted in increased magnitude, faster development and longer lasting duration of use-dependent plasticity under AMPH compared to the placebo session. These results document a facilitatory effect of AMPH on use-dependent plasticity, a possible mechanism mediating the beneficial effect of this drug on functional recovery after cortical lesions.

Bihemispheric plasticity after acute hand deafferentiation 1170

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Abstract has not been submitted.

Understanding the Action of TMS on the Cortex: The Importance of Animal Studies 1171

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The use of TMS has enormously improved our understanding of the human motor system, but our knowledge of its actions upon the brain is still rudimentary. Animal studies are of unique importance here because they allow investigation of the fundamental mechanisms of TMS action and provide greater confidence for the correct interpretation of non-invasive studies in human volunteers and patients. Our laboratory has focused on the responses of individual corticospinal neurones to TMS: what proportion these neurones is excited, and are both fast and slowly-conducting neurones activated? We are particularly concerned with understanding the nature of the direct or D-response to TMS, as well as the far more complex indirect or I-wave components (I1, I2, I3 etc). I will discuss the relevance of these responses to the natural discharge of these neurones during voluntary movement. We have recently been investigating the role of cortico-cortical inputs in the generation of the later I wave responses. Activation of premotor areas can facilitate strongly I waves in corticospinal neurons in primary motor cortex (Shimazu, H., Cerri, G., Maier, M.A., Kirkwood, P.A. and Lemon R.N. Eur. J. Physiol, 2002, S307). These results have exciting consequences for understanding cortico-cortical interactions, and on mapping studies using TMS.

LTP and DC stimulation in rat motor cortex slices 1172

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It has long been known that electric field may modulate the excitability of nerve cells, however, the influence of the application of direct current (DC) on synaptic transmission has not been well explored. Neocortical and hippocampal pyramidal neurons have favorable geometry for the investigation of the effects of the electric field oriented parallel to the long, somato-dendritic axis of these cells in isolated slice preparation. Rat brain slices were incubated in a modified interface perfusion chamber, in which two wire electrodes were placed in on both sides of the preparation to impose the electrical field. In hippocampal slices the field oriented positive to negative from the soma to the apical dendrites induced hyperpolarization of somatic membrane and an increase in the number

of spikes evoked by of depolarizating current pulse. The opposite field orientation induced reverse effects on the pyramidal cell excitability. The effects of DC application on field potentials (FPs) evoked by stimulation of afferents were studied in motor cortical slices. The application of DC for 10 minutes resulted in an immediate change of FP amplitude, either an increase or a decrease, which apparently correlated with the strength and the polarity of applied DC. FPs evoked in superficial and deep cortical layers showed opposite changes in magnitude which usually outlasted the time of DC application. In about 20% of slices the duration of these effects exceeded 30 minutes, thus resembling long-term potentiation (LTP) and long-term depression (LTD). Ongoing experiments are aimed at finding whether DC application facilitates the induction of LTP by θ-burst stimulation.

1173 The Thought-Translation-Device (TTD): A brain-computer-interface for the completely paralyzed

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On-line classification of slow cortical potentials (SCP) of the EEG allows the characterization of two to four different brain states which can be used for computer based selection of letters or words. 7 completely paralyzed, artificially respirated and fed locked-in patients suffering from amyotrophic lateral sclerosis were trained to produce negative and positive SCP at the Vertex-electrode. After successful training selection of letters or words was possible with a maximum speed of one letter or word per minute. Patients whose training was initiated late at a completely locked-in-state were only able to answer yes-no questions with their SCP. New classification algorithms, in particular support vector machines, allow better classifications. First attempts to build a 150 channel MEG/EEG brain-computer-interface (BCI) for the locked-in and otherwise paralyzed patients are reported.

Critical for successful application, however, is the selection of simple behavioral paradigms allowing patients with cognitive disorders and higher animals to produce classifiable brain states. The operant regulation with feedback seems to be an easy to apply and psychologically most adapted paradigm for BCI.

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Safety aspects of transcranial direct current stimulation 1174

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Transcranial direct current stimulation (tDCS) is capable of inducing excitability changes in the human motor cortex lasting for up to one hour after the end of stimulation, so far. The direction of the excitability shifts is determined by the current polarity. The duration of the after-effects is controlled by the stimulation duration. According to currently available safety criteria and safety measures, the developed tDCS paradigm should be regarded as safe. However, safety data for extended tDCS paradigms inducing excitability shifts outlasting for days or weeks, which are needed for possible clinical applications, are limited. Two different experimental approaches were carried out:

To extend safety data for the currently available tDCS-protocols, MR imaging was performed in human subjects before, immediately after, 30 min and 60 min after tDCS protocols (9 and 13 min.), which are known to shift motor cortical excitability for about one hour. No pathological signal alterations were detected with regard to apparent diffusion coefficients and contrast enhanced imaging. These results again attest the safety of the applied tDCS-protocols.

To determine the safety limit of extended tDCS protocols, a tDCS rat model was established with the stimulation electrode (3.5 mm^2) fixed onto the skull above the frontal cortex and a large, second electrode mounted onto the chest of the animal. In a preliminary sequence of escalating cathodal DC stimulations, intensities of 1, 10 100, 500 and 1000 µA were applied for 15, 30, 90 and 270 minutes in this rat model. According to the histological and immuno-histochemical evaluation, a threshold at a current density of about 140 $\mu A/mm^2$ could be determined for the induction tDCS-related brain damage. With respect to the size of the stimulation electrode (3.5 versus 3500 mm^2) this threshold intensity is about 500 times higher than the current density so far applied in the human. Analyses of 3-D reconstruction of induced cortical lesions revealed a linear lesion-size/coulomb function. Above the threshold of 0.2 coulomb, the volume of the DC-induced lesion is multiplied by 2.8 when the applied electric charge applied is increased by 1 coulomb.

The outcome of this study suggest that prolonged stimulation protocols could be established without causing harmful effects, at least for cathodal tDCS. However, since electrode positions, thickness of bone and cortical architecture differ between species, additional studies in animals, which are more similar to the human have to be performed before the results can be adapted to develop prolonged stimulation protocols in humans.

Inducing LTP and LTD like effects in the human motor cortex 1175

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Weak transcranial direct current stimulation (tDCS) induces excitability changes of the human motor cortex during stimulation, which can outlast the stimulation for minutes if

stimulation strength and duration are sufficient. Anodal stimulation enhances excitability, whilst cathodal stimulation diminishes it. Duration of the effect depends on stimulation duration and current intensity. We were interested if a further prolongation of stimulation duration could prolong the duration of the after effects. Furthermore, we studied the cortical level of the excitability changes by comparing measures of MEPs elicited by TMS, transcranial electrical stimulation (TES), H-reflexes, and TMS double stimulation. Anodal and cathodal tDCS of the motor cortex was applied for 5-13 minutes in separate sessions of a repeated measures design in 12 healthy subjects. Amplitudes of MEPs were measured before and up to 150 minutes after the termination of tDCS every fifth minute. Additionally the effects of tDCS on TMS-elicited MEPs were compared to those on TES-elicited ones, and to those on H-reflex amplitude. Moreover, the effects on intracortical inhibition and facilitation were tested by applying the double pulse TMS technique. While 5-7 minutes tDCS resulted in excitability changes lasting for a few minutes, longer stimulation durations elicited after effects lasting up to one hour after the end of tDCS. Anodal tDCS enhanced excitability, whilst cathodal stimulation diminished it. Neither TES-elicited MEPs nor H-reflexes were changed in amplitude. Anodal tDCS enhanced intracortical facilitation and reduced inhibition, while cathodal tDCS resulted in reversed effects. tDCS enables a modulation of motor cortical excitability, which lasts for up to an hour after the end of stimulation, if stimulation duration is sufficient. Because those excitability shifts are measurable by TMS, which monitors intracortical excitability, but not by TES and H-reflexes, both being indexes of excitability of the cortico-spinal tract, those changes are most probably localised intracortically. Moreover, tDCS has specific effects on intracortical inhibition and facilitation. Thus, tDCS is a promising new tool for inducing neuroplasticity in a non-invasive, selective, and focal way in the human motor cortex.

1176 Combining rTMS and DC stimulation of the motor cortex

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Long-lasting excitability changes can be induced in the human primary motor cortex (M1) by repetitive transcranial magnetic stimulation (rTMS) or by weak transcranial direct current stimulation (TDCS). Here we studied the interaction between these two interventions. With ethical permission, eight healthy subjects received anodal (1mA), cathodal (1mA) or sham (0mA) TDCS over the left primary motor hand area for 10min, followed by 15min of 1Hz rTMS at 90% resting motor threshold. Before and after TDCS and after rTMS, motor cortical excitability was evaluated by measuring the amplitude of surface EMG responses (MEPs) evoked by single pulse TMS in the right first dorsal interosseus muscle. TMS was applied through a figure-of-eight coil held perpendicular to the central sulcus. As reported previously, MEPs were increased after anodal TDCS but decreased after cathodal TDCS. When rTMS was given after sham TDCS, there was no consistent effect on MEPs. However, if rTMS was given after cathodal TDCS we found a lasting increase in MEP size, whereas if rTMS followed anodal TDCS MEPs were reduced. These findings suggest that short-term plasticity induced by

1Hz rTMS in M1 is scaled to the "functional state" of the cortex at the time of stimulation, and a preceding session of TDCS can be used to tune the cortical response to rTMS. This may be of relevance for the use of TDCS and rTMS as a therapeutic tool in patients.

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Pharmacology of tDCS 1177

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Recently it has been shown that weak direct current stimulation of the human motor cortex results in polarity-specific shifts of cortical excitability during and after the end of stimulation. Anodal stimulation enhances and cathodal stimulation reduces excitability. Animal experiments have demonstrated that the effect of anodal direct current stimulation is caused by neuronal depolarization, and cathodal stimulation hyperpolarizes cortical neurones. Here we show by pharmacological intervention in the human that the sodium channel blocker carbamazepine selectively eliminates the excitability enhancement induced by anodal stimulation during and after direct current stimulation. Blocking calcium channels by flunarizine results in similar modulations of the current-induced excitability changes, but the effect during stimulation is less clear-cut. Antagonizing NMDA-receptors with dextrometorphane does not alter current-generated excitability changes during a short stimulation, which elicits no after-effects, but prevents the induction of long-lasting after-effects independent of their direction, excitability enhancement or diminution. Amphetamine interacts selectively with anodal tDCS. We conclude that the cortical excitability shifts induced during direct current stimulation depend on membrane polarization, thus modulating the conductance of sodium- and calcium-channels, while the after-effects are NMDA receptor-dependent. Because the anodal after-effects are eliminated by the calcium channel blocker flunarizine, the respective NMDA-receptor modulation most probably depends on an increase of intracellular calcium level caused by membrane depolarization during stimulation. Amphetamine seems to be well-suited to stabilize the after-effects of anodal tDCS.

TMS in Clinical Neurophysiology 1178

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The standard motor evoked potentials technique (MEP) following single pulse TMS assess the rapidly conducting cortico-spinal and cortico-bulbar routes and have proved a sensitive diagnostic method in suspected central motor involvement. The sensitivity of MEPs was enhanced by recording from several target muscles and evaluating, in addition to latencies and amplitudes, extra parameters such as potential duration and variabi-

lity. The major draw-back of non quantifiable amplitude reduction can now be overcome by the triple stimulation technique, which considerably improves the diagnostic yield. In spite of their great sensitivity in multiple sclerosis (MS), MEPs usually do not enhance the diagnostic certainty in suspected MS, where MRI, CSF, and visual evoked potentials are more powerful diagnostic tools. In contrast, MEPs have proved helpful in the diagnosis of spinal cord disorders such compressive myelopathy, in ALS, multisystem atrophy, and in psychogenic paraplegia. MEPs are also a useful screening method in doubtful clinical signs of cortico-spinal tract involvement. In multifocal motor neuropathy they can identify a very proximal conduction block which is otherwise not accessible to non-invasive electrodiagnostic testing. MEP have also proved useful in monitoring motor abnormalities and the recovery of motor function, and they are of prognostic help in stroke. Apart from standard MEPs, numerous additional TMS applications have been developed: Assessing of motor cortex thresholds, TMS induced postexcitatory contralateral and ipsilateral silent period, paired-pulse stimulation techniques, input-output curves, and peri-stimulus time histograms with needle recordings. While these techniques give very useful information on the pathophysiology of the processes, they are not yet sufficiently standardised to be used as routine diagnostic tool. Likewise, repetitive stimulation techniques have not yet proved rewarding for diagnostic purposes. Exciting peripheral nerves by TMS is routinely done for motor root stimulation at the neck to assess peripheral conduction time in the MEP procedure and for intracranial (i.e. canalicular) facial nerve stimulation in the neurography of this cranial nerve. In these situations a circumscribed and stable stimulation site is set by the surrounding tissue, which is not the case for other deeply lying nerves. Canalicular stimulation has thus proved a valuable extension of facial neurography. However, by virtue of its particular stimulating mechanism it follows special rules which must be known to properly interpret the results.

Apart from rare seizures, no immediate or delayed severe adverse reactions have so far been reported with single pulse stimulators when necessary precautions are considered and the technical safety standards are met.

1179 Affections of the cortical silent period in motor disorders

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A silent period among a background of EMG activity can be produced by stimulating the motor cortex with magnetic or electrical stimulation. It normally appears after a motor evoked potential and lasts somewhat longer than the peripheral silent period which can be induced by supramaximal electrical stimulation to peripheral nerves innervating a tonically contracted muscle.

The silent period after transcranial magnetic cortical stimulation is thought to be composed of an early partially spinally induced and a late exclusively cortically induced component. It shares some pharmacological and neurophysiological features with the long interval intracortical inhibition. It has been suggested that activation of postsynaptic GABA-B receptors plays a role in generating the cortical silent period, and that it is induced locally within the primary motor cortex although recent evidence raises the

possibility of a contribution also from the ipsilateral somatosensory cortex and motor areas different from the M1 representation of the target muscles.

An abnormal short or long silent period can be observed in various diseases when the excitatory or inhibitory modulation of the local cortical interneurons is disturbed due to degenerative or ischemic lesions of various afferent systems. Especially, in ischemic lesions of the primary motor cortex a dominant interneuronal lesion can result in decreases or loss of silent period at preserved motor evoked potentials. Such an abnormality seems to be also of importance for symptomatic epilepsy in a subgroup or stroke patients.

Motor cortex excitability in chorea and myoclonus 1180

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Brain excitability in chorea and myoclonus has been mainly assessed by studies with the cortical silent period (CSP) and with the cortico-cortical inhibition (CCI).

CSP is generally prolonged in most patients with Huntington's disease (HD). This finding originally suggested a dysfunction of cortical inhibitory mechanisms in HD. But, more complex --and less specific-- mechanisms probably contribute to this abnormality. In HD the impairment of intracortical tangential flow of information and of cortico-cortical excitatory connections, the abnormalities of thalamo-cortical circuit, and, finally, neurpsychologic abnormalities impairing the prompt resumption of voluntary contraction can all play a role in CSP prolongation. CCI studies in HD provided variable results depending upon the conditioning-test intervals tested and whether subjects were tested at rest or during voluntary contraction. Studies during slight voluntary contraction and testing long conditioning-test intevals failed to disclose CCI abnormalities in HD. Taken toghether CSP and CCI studies would exclude a primary consistent dysfunction of cortical inhibitory circuits as a pathophysiological clue in this condition.Transcranial brain stimulation studies have no consistent clinical application in HD.

CSP and CCI studies in myoclonic disorders provided variable results depending upon the specific aethiology of the condition. Despite the limited clinical utility, CSP and CCI studies in myoclonic disorders provide a potentially useful tool for testing the efficacy of antimyoclonic drugs in humans.

TMS and tremor 1181

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Abstract has not been submitted.

1182 TMS and cerebellum

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TMS was used to study postexcitatory inhibition as well as intracortical inhibitory and excitatory phenomena in patients with cerebellar ataxia. MEPs following single and paired TMS were recorded from the first dorsal interosseus muscle (FDI) in 15 patients with autosomal-dominant or idiopathic cerebellar ataxia and 15 age matched normal controls. Mean postexcitatory inhibition was prolonged in the patient group. MEP amplitudes after paired TMS with short interstimulus intervals (1-4 ms) showing intracortical inhibition in the control group were not significant different in the patient group. In contrast, with longer interstimulus intervals (8-20 ms) mean MEP amplitudes were significantly reduced in the patient group, indicating a decrease of intracortical facilitation. Our findings support the idea that the cerebellum physiologically exerts a facilitatory influence on the motor cortex which is decreased in patients with a cerebellar degeneration.

1183 TMS in ALS

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Abstract has not been submitted.

1184 The effect of rTMS on sensorimotor function and focal dystonia

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It has been reported that 1 Hz subthreshold rTMS over the lateral premotor cortex (LPMC) causes inhibition of the primary motor cortex (M1), but those studies employed MEP for evaluating the excitability of M1 (Gerschlager et al, 2001; Munchau et al, 2002). Therefore, we studied the effect of TMS over the LPMC on M1 during voluntary finger movement in human (Chen et al, in press). In 8 normal subjects, EEG and EMG were simultaneously recorded during voluntary contraction of the right thumb before and after a 15 min train of 0.9 Hz rTMS over the left LPMC at stimulus intensity of 90 % of active motor threshold. After-effects on cortical motor activity were assessed by measuring the task-related EEG power and interregional coherence changes, and the

EEG-EMG coherence. As the results, the task-related power decrease in the α and β bands was reduced, and the EEG-EMG coherence decreased after the rTMS. These findings suggest a transient reorganization of the movement-related neuronal activity in the M1 after 1 Hz rTMS over the LPMC.

In writer's cramp, a line of evidence suggests reduced intracortical inhibition resulting in over-activation of M1 as well as non-primary motor areas. We studied 9 patients with writer's cramp and 7 age-matched normal subjects using subthreshold 0.2 Hz rTMS over the left LPMC (Murase et al, unpublished data). Stimulation of LPMC, but not of M1, significantly improved the rating of handwriting evaluated as the mean tracking error from the target, and prolonged the silent period in the patient group. rTMS over the supplementary motor area (SMA) did not affect the silent period, but significantly improved the handwriting though to a lesser degree as compared to the LPMC stimulation. These findings suggest that lack of inhibition in M1 may be secondary to hyperactive LPMC neurons, and the inhibition of LPMC by rTMS could provide a therapeutic measure in writer's cramp.

Triple stimulation technique (TST): Clinical applications 1185

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Motor evoked potentials (MEPs) are usually smaller in amplitude than responses to maximal peripheral nerve stimulation, and vary markedly between subjects and from one stimulus to another. Consequently, MEP amplitude measurements have low sensitivity to disclose central motor conduction failures due to the broad range of normal values. These characteristics result from varying desynchronization of the descending action potentials, causing varying degrees of phase cancellation. The TST, described in detail earlier, allows resynchronization of the MEP, and thereby a quantification of the proportion of motor units activated by the transcranial stimulus. In healthy subjects, the TST demonstrated that nearly 100% of the motor neurons supplying the target muscle can be brought to discharge.

In clinical studies, the TST has several advantages:

1) Increased sensitivity: In a large study including 271 unselected patients with a variety of central nervous disorders, the TST was 2.75 times more sensitive than conventional MEPs in disclosing cortico-spinal conduction failures (Magistris et al. 1998). In amyotrophic lateral sclerosis (ALS), the sensitivity increased by 2.2 fold (Rösler et al. 2000). Combining the TST to upper and lower limb muscles further increased the sensitivity by 1.5 fold (Bühler et al. 2001).

2) The TST introduces a quantitative measure of central motor conduction failure caused by conduction block, inexcitability, or loss of upper motor neurons. Thus it complements the conventional measurement of central conduction times. In all diseases studied so far, central conduction failures were more common than increased central conduction times (Magistris et al. 1999). The size loss of the TST response paralleled the muscle weakness in patients with purely central nervous diseases. In ALS, the TST often demonstrated central conduction failures, which were clinically obscured by simultaneous

loss of lower motor neurons. However, central conduction failure was usually less pronounced than peripheral loss of axons (Rösler et al. 2000).

3) Quantification of conduction failure offers an unambiguous means for patients follow up. For example, a marked improvement of conduction could be demonstrated in 50 patients with multiple sclerosis (MS) during a 5 days treatment with dexamethasone (Humm et al. in preparation). We envisage using the method in controlled treatment trials.

4) The TST allows insights into mechanisms of abnormal cortico-spinal conduction. For example, when compared with conventional MEPs, it allows an assessment of the degree of central desynchronization (Rösler et al. 2002). Interestingly, in MS; desynchronization is far more pronounced in patients with the chronic progressive form of the disease than in those with acute MS (in preparation).

Summarized, the TST is a helpful tool with considerable impact on diagnostic certainty in many patients. It will be suited to follow the disease course with or without therapeutic interventions, and will increase our knowledge about the conduction abnormalities in disorders of the cortico-spinal tract.

1186 Interhemispheric inhibition in stroke

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Adult brain has the ability to reorganize and adapt to compensate for ischemic injury. The mechanisms of reorganization may include recruitment of ipsilateral motor projections and changes in motor cortex excitability in the affected and undamaged hemisphere. Less is known about the influence of transcallosally mediated interactions between both motor cortices on such neuroplastic changes. Here we review recent research using transcranial magnetic stimulation (TMS) in study neuroplasticity in the intact motor cortex after acute stroke. To characterize the influence of interhemispheric interactions patients with monohemispheric cortical and subcortical stroke were investigated. Interhemispheric inhibition (syn. transcallosal inhibition; TI) was measured by applying TMS to the motor cortex resulting in inhibition of contralateral motor cortex and suppression of tonic voluntary EMG activity in hand muscles ipsilateral to TMS. In patients with ischemic lesions including the motor cortex (cortical group) TMS did not elicit inhibition of the contralateral motor cortex, while patients with stroke lesions caudal to the corpus callosum (subcortical group) showed normal interhemispheric inhibition. Ipsilateral motor responses indicating corticospinal reorganisation did not occur in any patient. Paired pulse TMS paradigm was performed to assess cortex excitability (intracortical inhibition and facilitation) in both groups of patients. In patients with cortical lesions and lacking interhemispheric inhibition an abnormal disinhibition of the unaffected hemisphere was measured. Patients with intact interhemispheric inhibition showed normal intracortical inhibition and facilitation in the motor cortex of the undamaged hemisphere.

These findings demonstrate a strong relationship between interhemispheric inhibition and cortex excitability in patients with acute stroke. Disruption of transcallosal modulation is associated with hyperexicitabilty of the undamaged hemisphere. It remains to be

clarified whether cortical disinhibition plays a significant role in functional recovery after stroke.

Magnetic stimulation and brainstem 1187

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Transcranial magnetic stimulation (TMS) of the cortical areas supplying cranial nerve innervated muscles has several useful clinical applications. Recordings from the facial muscles and the tongue are especially suitable to assess cortico-bulbar function due to the relatively large size of their cortical representation area. In contrast to limb muscles, fractionated examination of the projections to the facial muscles and the tongue require special recording and stimulation techniques which will be described in detail.

Central course of the cortico-bulbar pathways: Analysing the lesion topography of patients with unifocal infarctions affecting or sparing the cortico-facial and cortico-lingual projections we reconstructed the course of these pathways in the human brainstem. We could demonstrate that in the majority of subjects the cortico-facial fibres travel within the ventromedial base of the pons and cross the midline at the level of the facial nucleus. In some individuals, however, we found evidence that cortico-facial fibres form an 'aberrant bundle' in a paralemniscal position at the dorsal edge of the pontine base. In other patients the cortico-facial fibres loop down into the ventral part of the upper medulla, cross the midline and ascend in the dorsolateral medullary region ipsilaterally to the facial nucleus. The cortico-lingual fibres travel in close proximity to the cortico-facial projections and cross the midline at the level of the hypoglossal nucleus but we found no evidence for a paralemniscal position of cortico-lingual fibres.

Dysarthria: The cortico-lingual projections are frequently impaired in ischemic dysarthria. In a prospective study, including 106 patients with sudden dysarthria due to a single ischemia, we showed that in extracerebellar locations the cortico-lingual projections were affected in 91% while other potentially speech relevant projections were spared. Thus, in dysarthria without other localizing signs, TMS may contribute to lesion localization.

Multiple sclerosis: In patients with multiple sclerosis involvement of the cortico-bulbar projections was shown to disclose clinically silent lesions, supporting a spatial lesion dissemination.

ALS: The cortico-bulbar tracts are frequently affected in amyotrophic lateral sclerosis as observed in 51 consecutive patients with different clinical patterns of the disease. Transcranial magnetic stimulation to the cranial nerve muscle is useful to confirm the oftenly subclinical involvement of the upper motoneuron and to differentiate ALS from cervical spondylotic myelopathy.

Facial palsy: Fractionated examination of the cortico-facial pathways may also contribute to the diagnostic work-up of facial palsies. Even in incomplete Bell's palsy magnetic stimulation of the proximal portion of the facial nerve frequently shows an absent or amplitude reduced compound muscle action potential (CMAP) immediately after the occurrence of the paresis and thus differentiates from a supranuclear or intraaxial infra-

nuclear lesion site. In peripheral facial palsies due to borreliosis or malignant meningeosis the contralateral facial nerve is often subclinically affected. Facial paresis due to Guillain-Barré-syndrome show prolonged latencies after cortical stimulation and proximal stimulation of the facial nerve.

1188 TMS in Stroke

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Objective: To investigate the effects of circumscript brain lesions on motor (cortex) excitability.

Methods: A combination of various TMS techniques was employed to study changes of motor excitability in stroke patients with well defined brain lesions. Intracortical inhibition (ICI), intracortical facilitation(ICF), stimulus response curves (SRC) and silent periods (SP) were measured.

Results: Somatosensory cortex lesions were associated with a decrease of ICI, an increase of ICF and a shortening of the SP. A lesion in the territory of the superior cerebellar artery resulted in a loss of ICF, an increase of ICI and a suppression of SRC. Primary motor cortex lesions showed an intracortical disinhibition, combined with a suppression of SRC. In embolic striato-capsular lesions without involvement of the pyramidal tract ICI was reduced and SRC were enhanced.

Conclusions: The results may serve as a model to understand the interactions of various brain areas in normal subjects.

1189 The role of early visual areas during action observation.

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Recent neurophysiological studies have shown that perception and execution of actions are closely linked. Single cell recordings in monkeys and fMRI studies on humans have proved that premotor neural systems are not only activated during action execution, but also during action observation. These neuronal populations have been named "mirror neurons" to illustrate their behaviour. Transcranial magnetic stimulation studies have indicated that the excitability of the corticospinal system is facilitated during action observation. In the present study, we examined the hemianoptic patient GY, whose ability of blindsight has been proven in many experiments on blindsight. He is a 46-year-old male who sustained damage to the posterior left hemisphere of his brain by head injury when he was 8 years old. The lesion compromises almost all of his left striate cortex (V1) and small parts of his surrounding extrastriate cortex (V2 and V3). We used TMS to investigate whether the presentation of movements in his blindfield

would increase the amplitudes of motor evoked potentials (MEPs) or would facilitate his corticospinal system, respectively.

We found that G.Y. showed the same facilitatory effect compared to 8 control subjects during the presentation of action in his unimpaired visual hemifield. On the contrary, MEPs did not show a facilitatory effect while he observed motor actions in his blindfield. This finding may argue for the fact that the processing of visual information in early visual areas (V1,2) plays a crucial role for motor faciliation during action observation.

Transcranial direct current stimulation modulates the excitability of the somatosensory cortex **1190**

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The impact of transcranial direct current stimulation (tDCs) on the excitability of the somatosensory cortex was studied by analyzing the low frequency somatosensory evoked potentials (N20) and the high frequency oscillations (HFOs) under median nerve stimulation before and post tDCs.

The source activity of the N20 and HFOs was evaluated in ten healthy volunteers after 9 minutes of anodal and – in a second session - after 9 minutes of cathodal tDCs of 1mA and was then compared to the source activity before tDCs. We showed a significant decrease of N20 source amplitude after cathodal stimulation for about 20 minutes while HFOs were not affected. There was no influence of anodal stimulation.

Corresponding to previous studies concerning motor cortex the excitability of the somatosensory cortex can be reduced by cathodal tDCs. An increase of excitability as it was also shown for the motor cortex by anodal tDCs could not be found. Furthermore there is a dissociation of high and low frequency somatosensory evoked potentials which points to different generators. It is widely accepted that N20 is generated by excitatory postsynaptic potentials (EPSP) of pyramidal cells in area 3b whose excitability can apparently be reduced by cathodal tDCs. HFOs arise from oscillatory activity either in cortical interneurons or in thalamocortical projection fibers. This activity could not be inhibited or aggravated by tDCs.

1191 The role of the prefrontal cortex in verbal episodic memory: RTMS evidences

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In a previous study (Rossi et al., 2001) we used repetitive transcranial magnetic stimulation (rTMS) to interfere with left or right dorsolateral prefrontal cortex (DLPFC) during encoding and retrieval of pictures. The left DLPFC was involved during encoding while the right DLPFC during retrieval, in agreement with the HERA model (Tulving et al., 1994).

Unpublished post-hoc observation on the same task revealed that, interference by rTMS occurred only in subjects naive to visual stimuli, suggesting that the prefrontal cortex could be engaged only when novel information must be stored or remembered. In order to assess this observation, we apply the same paradigm to memory for semantically related or unrelated word pairs. During the encoding phase (study phase) twelve volunteers judged the relatedness of 8 related and 8 unrelated word pairs, according to norms collected for this experiment. One hour later (test phase), they were presented with the first word of each pair, and a choice between the second (old word) and a distractor (novel word). Trains of 10% subthreshold rTMS (500 ms, 20 Hz) were delivered, simultaneously to word presentation onset, to the DLPFCs (Brodmann area 9). The encoding/retrieval blocks were six: R-Enc (right rTMS in encoding, no stimulation in retrieval); L-Enc (left rTMS in encoding, no stimulation in retrieval); Sham (left rTMS in encoding and right in retrieval); R-Ret (no stimulation in encoding and right rTMS in retrieval); L-Ret (no stimulation in encoding and left rTMS in retrieval); Baseline, which served as reference condition, consisted of absence of stimulation in encoding and retrieval.

In the study phase the error rate was very low. On contrary, in the test phase error rate was significantly higher for R-Enc, L-Enc and R-Ret than all the other conditions, but only for unrelated word pairs. These results indicate that both DLPFC are involved during verbal encoding, while right hemisphere prevalence is found during retrieval, only in the case of novel word associations.

The findings presented here underline the role of DLPFCs when novel information must be stored or recalled and their, to some extent, consistency with the HERA model.

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Effect of the low-frequency repetitive transcranial magnetic stimulation (rTMS) on the background EEG activity

1192

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Background: The repetitive transcranial magnetic stimulation (rTMS) has been repeatedly shown to be able to modulate excitability and activation of the cerebral cortex. However, very little is known about its effects on the ongoing cortical electrical activity ("background EEG"). In this study we wanted the examine whether low-frequency rTMS can influence the dominant EEG background activity (posterior α) if delivered close to its principal site of cortical expression (i.e. parieto-occipital cortex).

Method: Six healthy subjects were studied. Four trains of 250 TMS stimuli, with 1-minute inter-train intervals, were delivered at 1Hz and at phosphene threshold over parieto-occipital cortex (the side varied between subjects). The EEG was recorded, with both eyes closed and eyes open in alteration, from four bi-polar channels, F3-C3, F4-C4, P3-O1, and P4-O2. The power in the θ (5.4-7.3Hz), α1 (7.8-9.8Hz), α2 (10.3-12.2Hz), and β1 (12.7-14.7Hz) frequency bands was calculated. In two subjects additional control recordings were done. In one of the subjects, besides the posterior site, the rTMS (at motor threshold intensity) was also delivered over anterior cortical areas (between F3 and C3 electrodes) ipsilateral with the posterior stimulation. In another subject an additional recording was done with placebo coil positioned over the same posterior site as in 'real' stimulation.

Results: The EEG from eyes open recordings did not show any consistent changes in relationship with rTMS delivery. However, rTMS significantly increased the power in the α1 frequency band from the EEG recorded with eyes closed. The increase started immediately after the first train and lasted up to 30 minutes after rTMS. Even when adjusted for baseline differences, the increase in α1 power after rTMS was significantly larger at posterior than anterior sites and was significantly the smallest at the anterior site contralateral to the side of stimulation. No other examined frequency band displayed changes associated with rTMS delivery. No comparable changes were seen after either anterior or placebo rTMS.

Conclusions: Low-frequency rTMS is able to induce lasting modulation of brain background electrical activity near the stimulated site and in functionally connected cortical areas. The increase in α1 activity is thought to represent a more disengaged brain state and would be compatible with the decrease of excitability and/or increase of inhibition that occurs after the low-frequency (1Hz or less) rTMS of the motor cortex. The data support the potential use of low-frequency rTMS in disorders with hyper-excitable functional brain networks.

1193 Effect of repetitive transcranial magnetic stimulation of the visual cortex on spatial hearing

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It is a widely held view that the occipital cortex (OC) is a unisensory area solely concerned with visual functions. However, single-unit recordings in cats have indicated auditory spatial input into extrastriate regions of the OC [1]. Also, sound lateralization in patients with right-hemispheric OC lesions and hemianopia seems to be systematically shifted [2]. Moreover, neuroimaging studies showed activations of extrastriate areas of the right OC in blind human subjects during tasks that required sound localization [3]. In the present study, we made a first attempt to reveal the potential role of these areas in spatial hearing of normal human subjects. For this purpose, we employed repetitive transcranial magnetic stimulation (rTMS) of secondary visual areas of the right OC in combination with a simple psychophysical task of sound lateralization, which was performed immediately after the end of rTMS application.

Dichotic pure-tone pulses (duration 50 ms, frequency 1 kHz, sound-pressure level 70 dB), delivered via insert earphones, were used as auditory stimuli. Interaural time differences (ITDs) of the sound stimuli were varied between trials. Subjects indicated the intracranial position of the sound image with respect to the median plane of the head by pressing a “left” or “right” key [4]. Application of 1-Hz rTMS for 7.5 min induced a mean systematic shift in the lateralization of ITDs (with reference to the pre-TMS condition) by 6.5 µs (±3.2 µs SE; P=0.008) toward the side of rTMS. This shift was demonstrable only within 10 min after the end of the rTMS period. The acuity of ITD discrimination was unaffected. In an accompanying experiment, we also applied rTMS to the right superior temporal gyrus, which is known to be part of the "where" stream, conveying auditory spatial information to the posterior parietal cortex and dorsolateral prefrontal cortex [5]. Here we found a similar shift in sound lateralization (mean 10.8 µs, ±3.1 µs; P=0.008) that was, however, opposite (i.e., to the left). A supplementary control experiment, in which rTMS was delivered to the vertex, gave no hint of nonspecific effects of rTMS on the subjects´ performance.

While the OC has previously been shown to process auditory information only in blind persons, the present findings provide the first direct evidence of an involvement of the visual cortex in spatial hearing in sighted human subjects, and suggest a close interconnection of the neural representation of auditory and visual space. Since rTMS induced systematic shifts in auditory lateralization, but not a general deterioration, we propose that rTMS of the OC specifically affected neuronal circuits transforming auditory spatial coordinates by processing eye-position information, in order to maintain alignment with vision.

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Transcranial direct current (tDCS) stimulation induces outlasting excitability changes in the human motor cortex, as revealed by transcranial magnetic stimulation

1194

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Weak transcranial direct current stimulation (tDCS) induces excitability changes of the human motor cortex during stimulation, which can outlast the stimulation for minutes if stimulation strength and duration are sufficient. Anodal stimulation enhances excitability, whilst cathodal stimulation diminishes it. Duration of the effect depends on stimulation duration and current intensity. We were interested if a further prolongation of stimulation duration could prolong the duration of the after effects. Furthermore, we studied the cortical level of the excitability changes by comparing measures of MEPs elicited by TMS, transcranial electrical stimulation (TES), and H-reflexes. Anodal and cathodal tDCS of the motor cortex was applied for 5-13 minutes in separate sessions of a repeated measures design in 12 healthy subjects. Amplitudes of MEPs were measured before and up to 150 minutes after the termination of tDCS every fifth minute. Additionally the effects of tDCS on TMS-elicited MEPs were compared to those on TES-elicited ones, and to those on H-reflex amplitude. While 5-7 minutes tDCS resulted in excitability changes lasting for a few minutes, longer stimulation durations elicited after effects lasting up to one hour after the end of tDCS. Anodal tDCS enhanced excitability, whilst cathodal stimulation diminished it. Neither TES-elicited MEPs nor H-reflexes were changed in amplitude. tDCS enables a modulation of motor cortical excitability, which lasts for up to an hour after the end of stimulation, if stimulation duration is sufficient. Because those excitability shifts are measurable by TMS, which monitors intracortical excitability, but not by TES and H-reflexes, both being indexes of excitability of the corticospinal tract, those changes are most probably localised intracortically. Thus, tDCS is a promising new tool for inducing neuroplasticity in a non-invasive, selective, and focal way in the human motor cortex.

1195 Modulation of after-effects of transcranial direct current stimulation (tDCS) - generated cortical excitability shifts by application of the $GABA_A$-agonist lorazepam

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Recently it has been shown that weak direct current stimulation of the human motor cortex results in polarity-specific shifts of cortical excitability during and after the end of stimulation. Anodal stimulation enhances and cathodal stimulation reduces excitability. These effects evolve during stimulation, but remain for up to an hour after the end of tDCS. Animal experiments suggest that anodal tDCS depolarizes and cathodal stimulation hyperpolarizes cortical neurones. Recently it was reported that the short-lasting after-effects of tDCS critically depend on NMDA-receptor efficacy changes. However, not much is known about the role of the GABAergic system in tDCS-generated cortical excitability changes.

To test if the GABAergic system is involved in the short- and long-lasting excitability changes after tDCS, we applied the GABA-agonist lorazepam 2 h before tDCS. Transcranial magnetic stimulation (TMS) -elicited Muscle evoked potential (MEP) amplitudes were measured before and after tDCS-protocols known to induce short- and long-lasting motor cortical excitability changes.

With regard to both short-lasting and long-lasting after-effects induced by anodal stimulation, compared to placebo-medication, application of lorazepam resulted in a delayed, but then enhanced and prolonged excitability elevation. In contrast, lorazepam did not change the excitability diminutions elicited by cathodal tDCS.

The enhancement of the anodal after-effects is consistent with recent findings that excitatory synapses activated in an inhibited cortical network are strengthened to a greater extent compared to those in active cortical networks. The delay of the excitability enhancement in the lorazepam-condition is suggestive for a transient activation of the inhibitory GABAergic system by anodal tDCS that is facilitated by lorazepam and via recurrent inhibition by the tDCS-activated excitatory system, the latter temporarily overbalanced in the initial post-stimulation phase.

1196 Modulation of long-lasting after-effects of transcranial direct current stimulation (tDCS) - generated cortical excitability shifts by application of ion-channel blockers and NMDA receptor-antagonists

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Recently it has been shown that weak direct current stimulation of the human motor cortex results in polarity-specific shifts of cortical excitability during and after the end of

stimulation. Anodal stimulation enhances and cathodal stimulation reduces excitability. These effects evolve during stimulation, but remain for up to an hour after the end of tDCS. Animal experiments suggest that anodal tDCS depolarizes and cathodal stimulation hyperpolarizes cortical neurones. However, not much is known about the physiological mechanisms underlying those excitability changes in the human.

To learn about the processes involved in the long-lasting excitability changes after tDCS, we applied the sodium channel-blocker carbamazepine, the calcium channel-Blocker flunarizine and the NMDA-antagonist dextrometorphane 2 h before tDCS. Transcranial magnetic stimulation (TMS) -elicited Muscle evoked potential (MEP) amplitudes were measured before and after tDCS-protocols known to induce long-lasting motor cortical excitability changes.

Carbamazepine and Flunarizine selectively eliminated the excitability enhancement induced by anodal stimulation. In contrast, antagonizing NMDA-receptors eliminated both, the excitability enhancement induced by anodal and the excitability reduction induced by cathodal tDCS.

We conclude that the long-lasting cortical excitability shifts induced by prolonged tDCS need membrane polarization shifts as a necessary precondition, but the outlasting effects critically depend on subsequent changes of NMDA-receptor efficacy.

Phamacological modulation of membrane potentials and NMDA receptor efficacy shifts during and after transcranial weak direct current stimulation of the human motor cortex 1197

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Recently it has been demonstrated that weak direct current stimulation of the human motor cortex results in polarity specific shifts of cortical excitability during and after the end of stimulation. Anodal stimulation enhances and cathodal stimulation reduces excitability. Animal experiments have demonstrated that the effect of anodal direct current stimulation is caused by neuronal depolarization, cathodal stimulation hyperpolarizes cortical neurones. Here we show by pharmacological intervention in the human that the sodium channel blocker Carbamazepine selectively eliminates the excitability enhancement induced by anodal stimulation during and after direct current stimulation. Blocking calcium channels by Flunarizine results in similar modulations of the current-induced ecxitability changes, but the effect during stimulation is less clear-cut. Antagonizing NMDA-receptors by Dextrometorphane does not change current-generated excitability changes during a short stimulation, which elicits no after-effects, but prevents the induction of long lasting after effects independent of their direction, excitability enhancement or diminution. We conclude that the cortical excitability shifts induced during direct current stimulation depend on membrane polarization, thus modulating the conducance of sodium- and calcium-channels, while the after effects are NMDA receptor-dependent. Because the anodal after effects are eliminated by the calcium channel blokker Flunarizine, the respective NMDA-receptor modulation most probably depends on

an increase of intracellular calcium level caused by membrane depolarization during stimulation.

The transcranial application of weak direct currents (transcranial direct current stimulation, tDCS) to the human primary motor cortex is capable of eliciting intracortical excitability changes. The direction of those modulations depends on stimulation polarity: Anodal stimulation increases excitability, while cathodal stimulation diminishes it (Nitsche and Paulus, 2000). The respective changes evolve during the stimulation but remain so far for up to an hour after the end of stimulation, given sufficiently long stimulation duration (Nitsche and Paulus, 2000, 2001; Nitsche et al., in press). Beyond those transcranial magnetic stimulation- (TMS) assessed electrophysiological changes, an fMRI study revealed similarly directed tDCS-elicited changes of motor cortical activity (Baudewig et al., 2000). Hereby, the efficacy of tDCS is not restricted to the motor cortex (Antal et al., 2001). Functionally, tDCS modulates use-dependent neuroplasticity as well as implicit motor learning (Nitsche et al., in press; Rosenkranz et al., 2000).

However, little is known about the underlying mechanism of the effects of tDCS in the human. In the animal during anodal stimulation cortical neurons are depolarized at a subthreshold level, while they are hyperpolarized by cathodal DCS (Purpura and McMurtry, 1964). Taking for granted that the respective membrane potential changes serve as a necessary precondition for the induction of after effects, the fact that the voltage dependent sodium channel blocker Carbamazepine (CBZ) eliminates the short lasting after effects induced by anodal, but not by cathodal stimulation indicates that this could be similar in the human (Liebetanz et al., 2002). However, the involvement of sodium channels in the effects of tDCS during tDCS has not been tested so far. Moreover, it is unknown if additional ion channels participate in tDCS-elicited excitability changes. Calcium channels are likely candidates, since in the animal the intracellular calcium level is increased after anodal DCS (Islam et al., 1995) and changes of intracellular calcium level are important for the induction of neuroplasticity (Bennett, 2000). Moreover, modulation of calcium channel activity could change the amount of transmitter release and thus modify cortical excitability. On the receptor level, NMDA-receptor modulation seems to be a necessary condition for the induction of short-lasting after effects of tDCS in the human (Liebetanz et al., 2002), which is of special importance because those are involved in neuroplastic mechanisms (Bennett, 2000). However, so far it is not known wether (i) NMDA-receptors are modulated even during short-lasting direct current stimulation, that per se does not induce after effects, and (ii) are of importance for the induction of long-lasting after effects elicited by prolonged tDCS.

Therefore, in the present study we tested

(a) the dependency of intra-current excitability modifications on changes of ion-channel conductivity by applying the sodium channel blocker CBZ and the calcium channel blocker Flunarizine (FLU);

(b) the involvement of NMDA-receptors in the generation of intra-current effects by antagonizing these receptors with Dextromethorphane (DMO);

(c) the dependency of long-lasting tDCS-induced after-effects on sodium and calcium channel activity as well as NMDA-receptor modulation by applying CBZ, FLU and DMO prior to tDCS protocols known to elicit long lasting after effects.

Effect of transcranial DC motor cortex stimulation on somatosensory evoked potentials in humans 1198

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Objectives: To study the effect of transcranial direct current stimulation (tDCS) over the left motor cortex on the size of somatosensory evoked potentials (SEPs) in humans.

Methods: SEPs were repeatedly recorded following motor threshold electrical stimulation of the right or left median nerve at the wrist at 3 Hz before and after anodal or cathodal tDCS in 8 healthy subjects (6 men and 2 women, 29-49 years). SEPs were recorded from scalp electrodes 2 cm posterior from C3 or C4 (parietal components: P14/N20, N20/P25, P25/N33 and N33/P40) and 5 cm anterior from C3 or C4 (frontal components: N18/P22, P22/N30) referred to the contralateral earlobe. tDCS was applied for 10 minutes to the left motor cortex at a current strength of 1 mA. Surface sponge electrodes (35 cm^2) were used for tDCS. One electrode was placed over the representational field of the right abductor pollicis brevis muscle, as determined by transcranial magnetic stimulation, was the other electrode placed above the contralateral orbita. The polarity refers to the electrode of the left motor cortex.

Results: Amplitudes of P25/N33, N33/P40 (parietal components) and P22/N30 (frontal component) following right median nerve stimulation were significantly increased after anodal tDCS and this conditioning effect continued over 60 minutes after the end of tDCS, whereas P14/N20, N20/P25 (parietal components) and N18/P22 (frontal component) were not affected. All components following left median nerve stimulation were unaffected by anodal tDCS. Cathodal tDCS had no effect on SEPs evoked from stimulation of either arm.

Conclusion: tDCS can have a polarity-dependent effect on the cortical excitability. Anodal tDCS over the motor cortex can induce a long-lasting increase in the ipsilateral sensorimotor cortical excitability.

Effects of Transcranial Direct Current Stimulation on Memory during Sleep 1199

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Sleep enhances memory consolidation. Furthermore, hippocampus-dependent declarative memory benefits particularly from slow-wave sleep (SWS) during the first part of the night, whereas procedural memory not depending on hippocampal function benefits particularly from later periods of sleep, where rapid eye movement (REM) sleep is dominant. Transcortical direct current (DC) potential shifts also occur spontaneously during sleep revealing a pronounced shift towards negativity at sleep onset. During SWS this negative potential gradually decreases. Transcranial direct current stimulation

(tDCS) leads to intracerebral current flow and can transiently influence cortical excitability. Here, we investigated the ability to influence SWS associated memory formation by applying tDCS over the frontal cortex. The use of tDCS targeted at identifying a possible role of the DC negative potential associated with SWS for memory processing.

Eighteen human subjects participated in 2 experimental sessions (placebo, anodal tDCS) separated by at least 1 week. Prior to sleep tasks of declarative (paired word associates) and procedural memory (mirror tracing), attention (d2 test) and mood (adjective check list) were assessed. Subjects went to sleep at 23:00 h and were awakened after one NREM-REM sleep cycle (as determined by polysomnography). Performances were subsequently tested. Anodal tDCS was started at the onset of SWS in an intermittent fashion (on/off: 15s/15s) over a total of 30 min, current density was 0.26 mA/cm2.

Prior to sleep mean (±SEM) immediate recall of declarative memory did not differ between tDCS and placebo, 32.9 (±1.5) vs. 34.1 (±1.5) words, respectively. After sleep declarative memory performance increased by 2.7 (±0.60) words when tDCS had been applied but by only 0.89 (±0.76) words following placebo ($p < 0.01$). Attention score was also increased after tDCS, 21.9 (±7.0) compared to placebo, 5.6 (±6.4, $p < 0.05$). Analyses of variance indicated effects for two adjective categories: Following placebo high spirits decreased ($p < 0.01$) and introversion increased ($p < 0.05$), but both maintained their values following stimulation. Mirror tracing performance did not differ between conditions.

In conclusion, application of anodal tDCS during SWS enhanced declarative memory performance but not procedural memory, which has been shown previously to benefit selectively from REM sleep. Further studies have to clarify whether this effect is due to strengthening consolidation or a proactive influence of tDCS on retrieval processes. The latter type effect is supported by the improved attention at the time of retrieval after tDCS. Anodal tDCS may improve memory function by enhancing the negative transcortical potential naturally present during SWS.

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1200 Clinical improvement of CRPS symptoms after repetitive paraspinal cervical magnetic stimulation

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Introduction. Complex regional pain syndrome (CRPS) is a neuropathic pain syndrome that typically follows a noxious event affecting the distal part of an extremity. Aside from pain, accompanying sensory, autonomic, trophic, and motor symptoms are characteristic. Electrical spinal cord stimulation (SCS) is an established treatment for therapy-resistant pain syndromes and has been shown to also reduce pain in chronic CRPS. Some case reports have suggested that transcranial magnetic stimulation (TMS) can influence neuropathic and low back pain. The main advantages of TMS is that it is non-invasive and can therefore be applied to ambulant patients with less chronic pain. In this

pilot study we investigated the effects of high-frequency repetitive magnetic stimulation (rMS) on different symptoms of CRPS.

Methods. The following parameters were assessed before and after stimulation of six patients (5 females; 1 male) with acute and 1 woman with chronic CRPS type I of the hand: feeling of stiffness, pain at rest and force-dependent pain (both measured on a visual analog scale, 0 to 100 mm), allodynia (assessed by stimulation with von Frey filaments), grip force (measured with a dynamometer in kPa), minute motor activity, and active range of motion (AROM). The rMS (10 times, each series lasting 10 sec with 20 Hz at 120% of cervical motor threshold intensity) was applied at the cervical nerve roots (C7-C8) of the affected side. For a sham stimulation three patients were stimulated with definitely subthreshold intensity (5% of stimulator output).

Results. Immediately after rMS four of the seven subjects reported a decreased feeling of stiffness in the finger of the affected side (64 mm to 31 mm) and a decreased force-dependent pain (56 mm to 25 mm). Minute motor activity and AROM were improved in three patients. Allodynia was improved in four patients. Only the chronic-pain patient, who had marked allodynia, indicated a temporary increase of pain at rest during and after stimulation. This patient also showed an improvement of AROM and the feeling of stiffness. Subjective improvement lasted for at least 24 hours in all patients. Improvement could be reproduced in three patients by repeated stimulations. While all parameters remained unchanged after sham stimulation, all patients could feel the changed stimulation parameters.

Conclusion. These preliminary results suggest that rMS may alleviate pain and motor symptoms of CRPS.

Repetitive magnetic and functional electrical stimulation reduce spastic tone increase in patients with spinal cord injury 1201

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Introduction. The application of repetitive magnetic stimulation (rMS) at lumbar nerve roots or peripheral functional electrical stimulation was found to reduce spastic tone increase of the lower limbs in patients with spinal spasticity. Similar effects were also found when rMS was applied at the peripheral nerves in patients with cerebral spasticity. We compared both stimulation methods in patients with spinal cord injury.

Methods. Six patients (all with complete spinal cord injury) participated. The patients were divided into two groups: 3 patients without and 3 patients with spastic tone increase. All were stimulated with a series of rMS (10 times, each stimulation for 10 sec at 20 Hz and 120% lumbar stimulation motor threshold) applied at the lumbar nerve roots (L3-L4) on the more affected side. They were also stimulated by individual electrical stimulation training. The spastic tone increase was evaluated clinically with the Ashworth scale and mechanically with the pendulum test before and after magnetic as well as electrical stimulations. The peak velocity of the first swing measured with the pendulum test (in deg/sec) was used for analysis.

Results. Whereas the Ashworth scale showed no change after rMS and electrical stimulations in the group of patients without spastic tone, the pendulum test showed a decrease in peak velocity from a high pre-stimulation level to more normal values. In the other group the Ashworth scale showed a clinical reduction of spastic tone, and the peak velocity increased from spastic to more normal values.

Conclusion. The reduced values on the Ashworth scale and the increased velocity of the first swing in the pendulum test in patients with spastic tone increase after rMS and electrical stimulations indicate a reduction of spastic tone. In patients without clinical spastic tone increase, rMS and electrical stimulation led to a reduction in peak velocity and no changes on the Ashworth scale. This may indicate increased resistance during swinging and possibly therefore more muscle activity. All in all, our preliminary results suggest that not only functional electrical stimulation but also lumbar rMS can help to reduce spastic tone, but they also cause more muscle activity in patients without spastic tone.

1202 Facilitation of goal directed motor tasks and position sense by repetitive peripheral magnetic stimulation (RPMS) - physiological and clinical aspects

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Our goal is the rehabilitation of learned goal-directed hand and finger movements like reaching and grasping following localized brain lesions of vascular or traumatic origin. As known highly controlled fine skilled motor tasks especially manipulation and exploration seldom recover sufficiently. The rehabilitation has to achieve a reduction of spasticity and a facilitation of voluntary movement activity.

Our concept is based on an activation of a reorganization processes in the CNS (neuromodulation) by induction of proprioceptive inflow to the CNS physiologically corresponding to the lost inflow during active movements.

For the induction of proprioceptive inflow to the CNS we use the repetitive peripheral magnetic stimulation (RPMS) which depolarizes thick myelinated nerve fibres of the terminal sensorimotor branches. The proprioceptive inflow is generated in two ways: activation of mechanoreceptors of the stimulated muscles during the induced contraction and relaxation (adequate) and via the direct activation of the underlying sensorimotor afferences (inadequate).

Various components of an improvement due to RPMS-effect are clinically and experimentally investigated:

Spasticity independent of the level of origin can always be suppressed by RPMS (Ashworth scale). In an clinical experimental investigation with spastic paresis of finger and hand extensors a dramatic decrease of spasticity and a increase of voluntary activity could be demonstrated.

In disturbed goal-directed motor performances like reaching and grasping the regularity of the performed trajectory could be improved.

Besides the improvement of motor performances an improvement of cognitive functions could be found. To investigate the influence of RPMS on a pure cognition ability, we have analyzed the effect of RPMS on local tactile extinction in patients after right sided brain lesions. It could be found, that recognition errors of different tactile stimuli could be clearly reduced. To analyze the modifying effect of RPMS on spatial cognition, the position sense under static as well as the position sense during goal-directed pointing tasks with the index finger shows remarkable improvement following RPMS.

Since RPMS implicits also a vibration component the viscoelasticity and the tonic activity should be differentiated. Therefore the muscle stiffness around the elbow joint during alternating extension and flexion movements under relaxed state is investigated. This work shows a reciprocally modification following the conditioning stimulation.

We may assume that the induced inflow to the CNS projects via the fast conducting myelinated nerve fibres to spinal, supra spinal and cortical systems related to spasticity, goal-directed movements and spatial cognition. Hereby the muscle spindle activity (i.e. tonic component) could be of essential importance. In a PET study of eight patients it was shown that - due to RPMS conditioning - areas of the fronto parietal circuits are activated. Therefore we may assume that RPMS primarily facilitates the well-known parieto-frontal neuronal circuits involved in goal-directed controlled movements. On this long lasting neuromodulation on spinal and cortical level gaba- and glutaminergic systems maybe involved.

Technical approaches to induce and evaluate goal directed motor tasks and position sense due to repetitive peripheral magnetic stimulation (RPMS) 1203

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Our concept is based on the activation of reorganisation processes in the CNS. For this purpose the repetitive peripheral magnetic stimulation (RPMS) is used to induce active, physiologically oriented movements of reaching and grasping.

The presented poster shows a overview of the technical approaches to induce coordinated movements:

For nerve fibre depolarisation a high time and spatial varying magnetic field impulse is applied. This impulse is repeated with a physiologically oriented frequency (i.e. 20 Hz). To evaluate the different effects of the RPMS we apply up to 5000 stimuli during one experimental session. Since coordinated movements should be induced a much higher quantity of applied stimuli is necessary, therefore special cooled coils are developed.

To induce coordinated movements the main task is to develop a closed loop control structure for a single movement. With the information of a precise position sensor it is

possible to build up a control loop e.g for the index finger. In the view of control technology this control loop is nonlinear and time varying and cannot be treated with standard (linear) control methods. Hence the capabilities of system identification are used to take nonlinear characteristics and different parameters into account. Such parameters are muscle fatigue, spasticity, rest activity and recovery.

The described system identification should also be used to evaluate the therapeutic effort by differentiating between induced and voluntary movement components by including the EMG.

Besides this technical approaches for RPMS different experimental approaches to evaluate the effects of RPMS are presented:

INDEX OF SPASTICITY. During a voluntary extension of the index finger the EMG of the index finger extensors and flexors as well as the displacement (goniometer) are registered.

VIDEO, EMG AND FORCE. To investigate the effect of RPMS concerning goal directed movements a coordinated registration of video data (20 pictures per sec.), EMG and grip-force (both up to 5000 samples per sec.) is developed. To achieve precise information of the different sensors a real time operating system is used to coordinate the sampling process.

COGNITION. The aim of this experimental setup is to investigate the improvement of deficiency in body scheme by RPMS. The position sense under static conditions and target movement by non visual control is registered. The difference between the angle of the elbow joint and the position of a reference point represents the accuracy of the subject's position sense.

MUSCLETONE. A torque motor is used to elicit a one dimensional passive movement of the relaxed forearm. The torque motor is integrated into a closed loop control of the angle of the elbow joint. To differentiate between viscoelasticity and tonic activity slow movements of 2.5 deg. per sec. are applied while the EMG of the biceps and the triceps is recorded together with the torque in the elbow joint.

1204 Long-lasting changes in corticospinal excitability after prolonged subthreshold 5-Hz repetitive transcranial magnetic stimulation (rTMS)

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Objective: The present study addressed two questions: (i) Are immediate after effects of subthreshold 5-Hz rTMS to the primary motor hand area (M1-HAND) on cortical excitability dependent on the number of stimuli per session? (ii) How long last these after effects?

Methods: In eight right-handed young subjects (22-30 years), two sessions of subthreshold 5-Hz rTMS were given to the left M1-HAND, using an eight-shaped coil. 150 stimuli were applied during session A, whereas session B consisted of 1800 stimuli. The order of sessions was counterbalanced across subjects. Stimulus intensity was set at 90% of resting motor threshold (rMT). Before and for 30 minutes after rTMS, we measured the amplitude of motor evoked potentials (MEPs), cortical silent period (cSP) short-latency intracortical inhibition (SICI) and facilitation (SICF). A repeated-measures ANOVA was used for statistical analsis ($p < 0.05$).

Results: 1800 stimuli of subthreshold 5-Hz rTMS caused a significant increase in MEP amplitude for at least 30 minutes, but there were no relative changes in SICI, SICF, and cSP. We found no changes in intracortical and corticospinal excitability after 150 stimuli of 5Hz rTMS.

Conclusion: Subthreshold 5Hz rTMS at 90% of rMT is capable of inducing a lasting increase in corticospinal excitability. However, a sufficient number of stimuli is necessary to produce a reliable facilitation.

Functional brain imaging combined with 1 Hz transcranial magnetic stimulation 1205

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Most brain imaging studies of the effects of cortical 1 Hz transcranial magnetic stimulation (TMS) have employed either positron emission tomography or single photon emission computerised tomography. We combined repetitive TMS and functional magnetic resonance imaging (fMRI) to investigate changes in brain activity after slow TMS over left dorso-lateral cortex. 10 subjects received 15 min of 1 Hz TMS at 90 % resting motor threshold ending 1-2 min before they performed a motor learning task. A control group of 14 subjects performed the same task during brain scanning without preceding TMS. Blood oxygenated level dependent signal changes during a 10 min long task performed after the delivery of TMS were measured in all subjects using fMRI. Analysis of the brain-imaging data revealed several differences in brain activation between the two groups, including TMS specific activation changes in prefrontal cortex. The study shows that the effects of 1 Hz TMS can be imaged using fMRI to reveal short term changes in task-related cortical networks.

1206 Modulation of Top-down Attentional Control by 'Virtual Lesions' of Posterior Parietal Cortex: Combining Repetitive Transcranial Magnetic Stimulation and Bundesen's Computational Theory of Visual Attention

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It has often been suggested that posterior parietal cortex (PPC) contributes to top-down control of visual attention, but based only on indirect evidence to date. To seek more direct evidence, we employed 10Hz repetitive transcranial magnetic stimulation (rTMS) over right or left PPC (or none) while participants performed partial-report tasks, reporting digits in a relevant color but not those in an irrelevant color from brief masked displays of one or two items. Relative to left PPC rTMS or none, right PPC rTMS affected performance for two-item conditions, interacting with the side of each target, whether two items were arranged in a row or a column, and whether the second item was in the target or nontarget color. Left PPC rTMS did not differ from no rTMS. Data were then analysed in terms of C. Bundesen's (1990) formal theory of visual attention, in which the efficiency of top-down control is operationally defined as the ratio of attentional weights for nontargets divided by those for targets. This revealed that right PPC rTMS enhanced the efficiency of top-down control for the ipsilateral right visual field, and diminished it for the contralateral field, particularly for displays with a row format (one item in each field concurrently). These results validate right PPC as one of the critical components in the attentional control network and indicate that rTMS over the right PPC is able to modulate the efficiency of top-down attentional control across space.

1207 The Role and Timing of Human Frontal Eye Field Involvement in Visual Search

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Convergent evidence from macaque single unit studies and human fMRI investigations implicate the frontal eye field (FEF) as part of the fronto-parietal system involved in covert visual selection. The responses of FEF neurons in macaques during discrimination of visual targets from distractors has led to the suggestion that FEF neurons form a visual salience map that identifies targets for potential saccades. However, little is known about the capacity of human FEF for visual analysis.

First we investigated the role of FEF in performance of single feature tasks and feature conjunction tasks. In both tasks, a 12-element stimulus array (2° visual angle) was presented for between 40 and 180 ms and followed by a mask until a response was made. Stimulus presentation duration for each task was determined by a staircase method to match subject performance at 75% accuracy. Subjects signalled the presence or absence of a target by a key press. Trials were presented without transcranial magnetic stimulation (TMS) or TMS delivered over FEF, vertex (a non visual control site), or V5 (a visual control site). Pulses were delivered at 10Hz for 500ms at 65% of stimulator output beginning with stimulus presentation onset. TMS did not disrupt performance on the feature task (d' no TMS = 2.1, FEF TMS = 2.4). Impairment of performance on the conjunction search task was seen only when TMS was delivered to the FEF (d' no TMS = 1.9, FEF TMS = 1.4) and was characterised by elevated false alarms at the expense of correct rejections. No eye movements or blinks were associated with TMS delivery. The dissociation of FEF stimulation effects on conjunction and popout search implicates this area in target selection.

Second we investigated the timing of FEF involvement in visual search. In three conditions TMS was applied over FEF for 500ms beginning at 0, 100 or 200ms post-stimulus onset, in a conjunction search task similar to that described above. Significant disruption of task performance was seen only in the 0 ms condition. In a follow-up experiment, we used a double pulse paradigm to specify more precisely the timing of TMS interference. TMS pulses applied at 40 and 80ms in the same trial significantly disrupted task performance. These data are in agreement with findings in the macaque showing that FEF activity during this time period predicts monkeys' behavioural reports on hit, miss, false alarm and correct rejection trials.

These findings de-couple stimulus processing from eye movement execution in human FEF and suggest that, as in the macaque, human FEF is important for target discrimination in cluttered visual arrays as well as showing a similar time frame of involvement.

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Transcranial direct current stimulation of the primary visual cortex modulates the amplitude of the N70-component of visual evoked potentials 1208

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Weak transcranial direct current stimulation (tDCS) induces prolonged excitability shifts in the human visual and motor cortex non-invasively and painlessly: Anodal tDCS enhances, while cathodal stimulation reduces excitability. The duration of the neural response to tDCS observed after the end of stimulation depends on current intensity and stimulation duration and was demonstated to last for up to 1 hour so far in the motor cortex.

The aim of this study was to test if tDCS could modulate visual evoked potentials (VEP) if applied over the primary visual cortex (V1). Thus, we applied cathodal/anodal tDCS (1mA) for 5,10, 15 min over the occipital cortex of 20 healthy human subjects and examined VEPs before, immediately after, 10 min, 20 min, 30 min and 40 min after the cessation of stimulation. For the 10 minutes condition we additionally tested different tDCS-electrode positions to learn about the optimum electrode montage. VEPs were evoked by high- and low-contrast sinusoidal grating patterns.

Anodal tDCS resulted in an increased amplitude size of the N70 component, while cathodal stimulation resulted in its diminution. The time course of the effects depended on tDCS-duration; prolonged stimulation resulted in longer lasting after-effects. tDCS did not change VEP latencies. These results were selectively achieved for the Oz-Cz-montage, other electrode montages did not result in any significant tDCS-generated VEP-shifts.

Our results show that tDCS induces prolonged alterations of VEP amplitudes, the direction and magnitude of the changes are stimulation-polarity and -duration dependent. Thus, tDCS of the visual cortex is capable of inducing prolonged cortical excitability changes and could evolve as a promising tool for studying and inducing neuroplastic mechanisms in this area.

1209 Increased REM density induced by anodal transcranial direct current stimulation over the left premotor cortex during posttraining REM sleep

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Introduction: One function of sleep is hypothesized to be the reprocessing and consolidation of memory traces. Using positron emission tomography, it was shown that during REM sleep in humans previously trained on an implicit motor learning there are cortical reactivations in the left premotor cortex, bilateral supplementary motor cortex and left posterior parietal cortex. Excitability elevation of the primary motor cortex induced by anodal transcranial direct current stimulation (tDCS) improves performance in the acquisition and early consolidation phase of implicit motor learning.

Objectives: We aimed to investigate if anodal tDCS over the left premotor cortex during post-training REM sleep period does influence REM sleep and memory processing.

Methods: 14 healthy, right handed, good sleepers (mean age: 36, range from 19 to 56 years) underwent complete polysomnographic recordings with one adaptation night and two nights of randomized anodal tDCS or placebostimulation. During the second and third polysomnographic night each subject, previously trained on a serial reaction time task (SRTT), received either 15 minutes of anodal tDCS or placebo stimulation over the left premotor cortex during the second REM sleep period. If the REM period was less than 15 minutes, a further tDCS was applied in the following period. 7 subjects were

wakened and retested immediately after stimulation, and 7 subjects were retested in the morning.

Polysomnography was scored according to standardized criteria. REM density was measured as the frequency of REMs (expressed in REM/30 second in each REM period). Comparisons between the nights in each period of REM sleep were performed using ANOVA and post hoc Tukey tests and separate ANOVA for SRTT for reaction time, errors and variability.

Results: The following main differences between stimulation night and placebo night were found: REM density was significantly increased in each anodal tDCS period in comparison with the corresponding placebo stimulation period ($p<0.04$ for 2.REM period, $p<0.01$ for 3. REM period, $p<0.05$ for 2.+3.REM period). Reaction times of the SRTT were significantly shorter immediately after stimulation (interaction of block and TDC and time period $p<0.02$ for absolute values $p<0.04$ for standardized values). There was no significant difference in sleep macrostructure data between both nights and for other SRTT reaction times values.

Conclusion: The high increase in REM density during anodal stimulated post-training REM sleep and lower reaction times immediately after tDCS, suggest that the brain areas reactivated during post-training REM sleep participate in the optimization of visuomotor response network, e.d. influenced by tDCS.

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Repetitive transcranial magnetic stimulation (rTMS) - influence on the brain metabolism

1210

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We investigated the influence of the 3 week series of high and low frequency rTMS on the basal brain metabolism in the group of patients with depression and obsessive compulsive disorder (OCD). In accordance with the neuroimaging data indicating that depressive disorder is connected with decreased brain metabolism, and OCD with increased frontal metabolism, we applied the low (1 Hz) frequency in OCD and high frequency (20 Hz) in case of depressive disorder. rTMS was applied with 100% of motor threshold measured by standard method using the EMG control. The high frequency was defined as 20Hz, 2 s train and 18 s intertrain interval (total number of stimuli 1800). The low frequency was defined as 1 Hz (total number of stimuli 900). The total number of rTMS sessions was 15. The psychopathology was measured by the psychometric scales (HAMA, HAMD, Y-BOCS, CGI).

18 fluoro-deoxy-glucose (18FDG) positron emission tomography (PET) was performed before and after the rTMS treatment. The statistical parametric mapping (SPM99) was used for voxel-to-voxel determination of the differences in 18FDG PET resting brain metabolism during the treatment.

We found that in depression the high frequency rTMS of the dominant hemisphere increased the brain metabolism in the prefrontal cortex. In OCD the low frequency rTMS decreased the brain metabolism in the region of application and also in basal ganglia. The changes of the metabolism reflected the improvement of the psychopathology in both groups. Our data indicate that rTMS is an effective treatment method in both diagnoses. The metabolic changes are similar to the effects of other treatment methods (pharmacotherapy) and the pathogenetic relevance is discussed.

This study was supported by the research project 00000023752 MZ CR and the project CNS LN00B122 MSMT CR.

1211 The influence of different frequencies of rTMS on Attention (Continuous performance test)

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An attention disorder is common in patients with frontal lobe pathology. High and low frequencies of rTMS should activate or inhibit stimulated cortex regions respectively, and rTMS is a very promising method in the treatment of attention deficit.

Our study investigates the influence of different stimulation frequencies on the outcome of the computer version of Continuous Performance Test (CPT II) in healthy volunteers.

12 healthy volunteers underwent rTMS localized on the dorsolateral prefrontal cortex (DPLFC) of dominant hemisphere during the performance of CPT. We tested the hypothesis that the low frequency worsens the attention parameters and the high frequency improves them.

rTMS was applied with 100% of motor the threshold measured by standard method using the EMG control. The high frequency was defined as 20Hz, 2 s train and 18 s intertrain interval (the total number of stimuli 1800). The low frequency was defined as 1 Hz (total number of stimuli 900). Stimulation took 15 minutes each. The double blind setting was provided by the shame synchronized alternative acoustic frequency. In case of the applied frequency of 20 Hz, participant simultaneously heard a frequency of 1 Hz in the same amount of dB (and vice versa).

Participants started to perform CPT immediately after the beginning of stimulation and the volunteers were stimulated during the whole CPT performance. The interval between the high and low frequency stimulations of each volunteer was at least 10 days in random order. The stimulation was always applied at the same time of day (within 1 hour) to avoid the influence of the circadial variation of the vigilance.

We observed the influence of different frequencies on attention and reaction time. The clinical and physiological relevance is discussed.

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Neurophysiological Evaluation of Visual Cortex Excitability in Blind Subjects using Image-Guided Transcranial Magnetic Stimulation 1212

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Purpose The present study was undertaken to systematically map the perceptions induced by focal and non invasive stimulation of occipital cortex in blind subjects. We have tried to address the following questions: (i) Is there variability in the properties of the perceptions as a function of the onset of the blindness? (ii) Which factors influence the number and attributes of the perceived sensations? (iii) Are there changes in the retinotopical organisation of phosphenes as a function of the degree of visual cortex deafferentation? Methods TMS was applied over the occipital cortex in blind subjects with some residual vision, blind subjects without any residual vision, congenital blind subjects and healthy controls. TMS was applied with a figure of eight coil to 28 positions arranged in a 2x2cm grid over the occipital area. A digitizing tablet connected to a PC computer running customised software was used to collect and analyse visual perceptions. A frameless image-guided neuronavegational device was used to describe the position of the stimulation coils relative to the occipital surface. Results Our results show that TMS is able to elicit visual perceptions in almost all sighted subjects and in some proportion of blind subjects. Quantitatively, phosphenes were more numerous by repetitive TMS and in short term blindness, but there were no differences in the characteristics of the visual perceptions as a function of the onset of blindness. Visual perception were more retinotopically organized in sighted compared with blind subjects. Conclusions We conclude that TMS in combination with image technologies could be useful to improve our understanding of the physiologic organisation of visual cortex in blind subjects.

Disruption of the neuronal circuitry subserving working memory, by low frequency repetitive TMS, using a visuospatial 3-back task: A negative study 1213

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The aim of the study was to investigate the functional anatomy of working memory by studying the effects of unilateral rTMS of the dorsolateral prefrontal cortex (DLPFC) on working memory (WM) using a visuospatial 3-back task.

14 normal volunteers underwent 10 minutes of low-frequency, sub-threshold stimulation. Stimulation was given in a randomised order to the right and left DLPFCs, vertex (Cz) and a sham stimulation to either the left or right DLPFC. During, and immediately after, subjects performed a visuospatial 3-back task.

There was no significant effect of rTMS on the performance of the task, or on reaction time when compared to sham stimulation.

The absence or rTMS effects on task performance may be an indicator of redundancy or compensation within the WM distributed network, enabling the task to be carried out whilst an area is being disrupted. The stimulation parameters chosen and focality of repetitive TMS may also have been contributing factors to the result.

1214 Exploring Baddeley's Phonologic Loop using transcranial magnetic stimulation

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Baddeley's model of working memory (WM) proposes a specialised verbal component: the phonologic loop- a buffer for incoming verbal information that will subsequently be acted upon by the central executive. The loop is comprised of two sub components: a *phonologic store* for the passive storage of verbal information, and an *articulatory control process* for the rehearsal of verbal information. The two components act together, in order to maintain verbal stimuli.

Neuroimaging has provided valuable evidence as to the possible anatomical location of these components. The inferior parietal cortex is the hypothesised location of the phonologic store, whilst Broca's Area the site of articulatory rehearsal. Neuroimaging, however, can only show the involvement of an area in a cognitive process. TMS, on the other hand, has the ability to establish the causal role and timing of the contribution of a given cortical region in a behaviour.

Functional dissociation between the phonologic loop and the articulatory control process has already been demonstrated. In order to anatomically dissociate the two processes, TMS will be given over the IPC and Broca's Area during conditions involving the observable effects of phonemic similarity and word length.

Spoken material can gain access to the phonologic store without articulatory rehearsal, where a discrete number of items can be stored in an abstract phonologic code. The phonemic similarity effect indicates the use of the phonologic store, where phonemically similar words are harder to recall than distinct words. The word length effect, on the other hand, is an operational indicator of the articulatory control process, where longer words become harder to recall than shorter ones.

The aim is to show the IPC is the site of the phonologic store, whilst Broca's area is responsible for the articulatory control process and also to show their interaction during the storage of phonologic material.

Transcranial magnetic stimulation of the prefrontal cortex during visuospatial working memory task performance 1215

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Working memory (WM) is the cognitive mechanism which enables humans to keep a limited amount of information active for a brief period of time. Evidence from lesion and neuroimaging studies point to the important role of the dorsolateral prefrontal cortex (DLPFC). The ability of transcranial magnetic stimulation (TMS) to create 'virtual lesions' has allowed researchers to attribute function to structure, in the manner of traditional lesion studies. TMS is safe, non-invasive, and can be performed in normal subjects in a repeated manner.

Subjects are given high-frequency, above-threshold repetitive TMS over the left and right DLPFCs during the performance of a visuospatial 2-back WM task. TMS pulse delivery is time-locked to the presentation of stimuli, in order to disrupt the 'on-line' active monitoring and manipulation processes required by the task. Mean accuracy and reaction times are recorded from each subject, and compared with control conditions.

Does chronic serotonin re-uptake inhibitor paroxetine treatment modulate human motor cortex excitability in healthy subjects? A TMS study. 1216

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Objective:

The aim of the present study was to assess the effects of long-term paroxetine treatment, a selective serotonin (5-HT) re-uptake inhibitor (SSRI), on cortical motor excitability, using Transcranial Magnetic Stimulation (TMS) in healthy subjects.

Background:

In vitro, serotonin produces facilitatory, disinhibitory and inhibitory effects on pyramidal output neurons. In humans, 5-HT stimulates motor functions (Jacobs & Fornal, Neuropsychopharm 1999;21:9S-15S) and it has been recently shown that single dose of SSRI like paroxetine or fluoxetine, increase executive motor areas activation in healthy subjects (Loubinoux I, NeuroImage 2002;15:26-36; JCBF, 1999;19:1365-75). This pharmacological-induced increase was correlated with improved motor performances in post-stroke patients (Pariente, Ann Neurol 2001;50:718-29). Efficiency and associated pathophysiological mechanisms of SSRI chronic treatment in stroke patients is under investigation. At the same time, the neurophysiological bases of drug action on motor cortical excitability has to be investigated in healthy subjects with TMS.

Design / Methods:

This randomized, cross-over, double-blind study was conducted on eighteen healthy subjects who received 20 mg daily paroxetine or placebo, respectively, over a period of 1 month separated by a period of 3 month wash-out. TMS was performed after each period of placebo/paroxetine treatment. To date, all subjects completed the study, data have only been processed for twelve subjects and the double-blind cannot still be broken.

TMS were applied through a focal figure-of-eight shaped coil (Magstim Co, Whitland, UK) at the optimum scalp position over the hand area of the left motor cortex to elicit motor responses in the controlateral First Dorsal Interosseus (FDI). Rest (RMT) and active motor thresholds (AMT), paired-pulse short interval inhibition (interstimuli interval (ISI): 2;3 ms) and facilitation (ISI: 10;12 ms), paired pulse long interval inhibition (ISI: 100;160 ms), MEP intensity curve, duration of cortical silent period, were investigated. In addition, spinal and neuromuscular excitability were also assessed by measuring duration of peripheral silent period, F waves and maximum rest and active M waves.

Preliminary results:

Short intracortical inhibition was significantly modulated by paroxetine at ISI 3 ms. Paired pulse facilitation and intensity curve seem also to be modulated by the drug although non significantly on 12 subjects. All other measures on MEP threshold, cortical silent period, paired pulse long interval inhibition, spinal and neuromuscular excitability remained non affected.

Conclusions:

From these preliminary results, it seems that long-term treatment of paroxetine acts on intracortical motor excitability. This study could represent the first step towards the better understanding of neural plastic changes induced by drugs acting on neurotransmission.

1217 Involvement of the primary motor cortex in mental rotation of hands: A TMS study

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Mental Rotation (MR) is sustained by a network of brain regions, including parietal, premotor and primary motor (M1) cortices. However it is still not clear whether M1 is involved in MR only of hands or of external objects as well. Here we report a TMS study in which the involvement of M1 in MR of hands and letters was tested. Ten healthy participants were asked to judge whether pairs (N=112) of line drawings depicting hands or letters were the same or mirror images. In half of the trials subjects had to compare pairs of stimuli with the same orientation (baseline condition), while in the other half they had to implicitly rotate the right stimulus of the pair (rotation condition).

There were three experimental situations: TMS of the primary left motor hand area delivered at 400 ms after stimulus onset, sham TMS and no-TMS. In all situations each stimulus pair was presented until verbal response (yes/no) 200 ms after a fixation cross. The results show a significant three-way interaction: experiment X stimulus type X condition. TMS slowed the RTs of the judgment task relative to hands in the rotation condition only. These findings suggest that MR of hands, but not of external objects, requires the involvement of M1.

Transcranial magnetic stimulation (TMS) and physiological regulation of slow cortical potentials (SCP) 1218

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Several studies have shown that a brain-computer interface (Thought Translation Device, TTD), controlled by self-regulation of slow cortical potentials (SCP), can contribute to communication of completely paralysed patients (Birbaumer et al., 1999; Kübler et al., 2001). However, some patients as well as healthy subjects have not been able to control their SCP even after extended neurofeedback training. Is there a possibility to support those non-learners in learning to control their SCP?

The main goal of our study is to explore whether it is possible to shift the SCP by means of transcranial magnetic stimulation (TMS) and hence to develop further methods in order to support the learning process.

Since our fMRI data show that negative SCP are accompanied by a significant activation of the supplementary motor area (SMA) and positive SCP by a significant deactivation of the SMA, we study the changes in SCP after low- and high-frequency repetitive transcranial magnetic stimulation (rTMS) over the SMA, known respectively for their inhibitory and excitatory effect on the cortex (Boroojerdi et al. 2000; Mottaghy et al. 1999).

Ten healthy volunteers were trained for four days with the TTD to self regulate their SCP.

The electroencephalogram (EEG) was recorded from the following positions against both mastoids: Cz, FC3, CP3, FC4, CP4. Training included 11 blocks on each of the four sessions, each block comprised 34 feedback trials. A trial consisted of a 2 seconds passive phase, in which no feedback was provided, and a baseline was recorded. An active phase followed lasting 3.5 s, in which visual feedback of SCP was provided as a cursor movement on a feedback screen. The cursor moved up and down proportionally to the current SCP amplitude compared to the previously recorded baseline (the algorithm is described in Kübler et al. 2001). In each trial participants had to move the cur-

sor towards the top (by producing a negative shift of their SCP) or towards the bottom of the feedback screen (by producing a positive SCP shift). The experimental design contained the following conditions: 1) feedback without rTMS, 2) feedback after high-frequency rTMS (15 Hz, 2 s.), 3) feedback after low-frequency rTMS (1 Hz, 30 s.), 4-5) feedback after high- and low-frequency sham (= placebo) stimulation. TMS was delivered over the SMA with a focal figure-of-eight magnetic coil (DANTEC MagPro). During sham stimulation the coil was placed at a 90° angle to the SMA.

RTMS had a differential effect on self-regulation of SCP: 15 Hz rTMS enhanced negative SCP and reduced positive SCP, whereas 1 HZ rTMS enhanced positive SCP but reduced negative SCP. These findings are in line with the notion that rTMS can be used to support locked-in patients in self-regulating their SCP.

Supported by the Deutsche Forschungsgemeinschaft and the Volkswagenstiftung

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1219 Determining the cortical stimulation site in TMS: Linking physiological measurements with physical field models

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We report a novel method to determine the site and size of stimulated cortical area in TMS. Applied to the motor cortex, it allows to determine the likely cortical representation of muscles. Up to now, the most common procedure for this is motor mapping. In motor mapping, the obtained two-dimensional distribution of coil positions with associated muscle responses is used to calculate a center of gravity on the skull. However, classical mapping does not allow to determine the exact stimulation site on the cortex and only rough estimates of its size are possible. Our method combines physiological measurements with a physical model used to predict the electric field induced by the TMS coil to overcome these limitations. In four subjects motor responses in a small hand muscle were mapped with 9 - 13 stimulation sites at the head perpendicular to the central sulcus in order to keep the induced current direction constant in a given cortical region of interest. Input-output functions from these head locations were used to determine stimulator intensities that elicit half-maximal muscle responses. Based on these stimulator intensities the field distribution on the individual cortical surface was calculated as rendered from anatomical MR data. The region on the cortical surface in which the different stimulation sites produced the same electric field strength (minimal variance 4.2 ± 0.8 %.) was determined as the most likely stimulation site on the cortex. In all subjects, it was located at the lateral part of the hand knob in the motor cortex. Comparisons of model calculations with the solutions obtained in this manner reveal that the stimulated cortex area innervating the target muscle is substantially smaller than the size of the electric field induced by the coil.

Dynamic course of intracortical interactions during recovery of motor function after stroke: A TMS paired-pulse study

1220

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Introduction: Recovery of motor function after stroke may depend on changes in motor cortical excitability and synaptic strength. Transcranial magnetic stimulation (TMS) of the motor cortex has been used to probe these changes. The motor response to a test TMS pulse may be modulated by a conditioning pulse applied before the test pulse (Kujirai 1993). Normally inter-stimulus intervals (ISI) of 3-5 mS are inhibitory and those of 10-15 are facilitatory. In patients with hemiparesis due to stroke, there may be alterations of the normal pattern in both the hemisphere most affected by the stroke (Liepert 2000; Manganotti 2002) and in the less affected (or unaffected) side (Liepert 2000; Shimizu 2002). The goal of the present study was to study paired-pulse modulation longitudinally over time in patients who had suffered a cerebral infarction affecting motor function.

Subjects & Methods: 23 subjects, all with a single cerebral infarction affecting movement of either hand were studied longitudinally, at <10 days post-stroke (visit 1), at 1 month (visit 2), and at 6 months (visit 3). 9 age-matched control subjects were studied at two times, at least one month apart. *TMS:* Motor evoked potentials (MEP) from the first dorsal interosseous muscle of each hand were recorded. A conditioning TMS pulse 80% of threshold strength was provided 3, 5, 10, or 15 mS prior to a test pulse that was 120% of threshold. Test stimuli without conditioning stimuli were interspersed. MEP from these control trials were used to normalize the others. Normalized MEP (nMEP) <1 represent net inhibition, nMEP >1, facilitation.

Results: Stroke patients and control subjects were similar in age and gender distribution. Grip strength and finger tapping rate increased over time, while the Fugl-Meyer scale, and the Functional Independence Measure increased principally during the first month.

Paired pulse inhibition at the 3 mS ISI occurred normally in the control subjects (nMEP: 0.62) as did paired-pulse facilitation at the 15 mS ISI (nMEP: 1.33). In stroke patients paired-pulse inhibition and facilitation also occurred normally in the affected hemisphere at the 1st (0.58, 1.55) visit. At the 2nd and 3rd visits the mean effect at normally inhibitory ISIs was neutral (nMEP close to 1), and facilitation occurred normally at 10 and 15 mS ISIs. On the *unaffected* side, there was also loss of inhibition, especially at the 2nd visit.

Conclusions: 1. Paired-pulse inhibition in the motor cortex decreases in the first few weeks after stroke, in both hemispheres. 2. Facilitation occurs normally at all times after stroke, implying that disinhibition may be partly responsible for recovery of motor function.

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1221 A temporally asymmetric Hebbian rule governing plasticity in the human motor cortex

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A conspicuous feature of synaptic plasticity established in diverse neuronal preparations is its dependency on the temporal order of the pre-and postsynaptic activity. It remains unknown whether this principle applies *in vivo*, in the adult motor cortex. Here we show that human motor cortical excitability can be up-or downregulated by pairing afferent volleys elicited by peripheral nerve stimulation with motor cortical stimuli, contingent on the timing of the cortical responses induced by the two stimulation modalities within near-synchrony. The physiological profile of the depression of cortical excitability, including the dependence on activation of N-methyl-D-aspartate (NMDA)- receptors and of L-type voltage-gated Ca^{2+}-channels, and that of the enhancement of cortical excitability, established previously, closely resemble what has been documented in reduced cortical preparations for long-term depression (LTD) or –potentiation (LTP), respectively. Taken together, these findings provide evidence for the in-vivo operation of a temporally asymmetric Hebbian rule governing the induction of LTD/LTP-like plasticity in intact human motor cortex.

1222 Involvement of long-term potentiation – like plasticity in human motor learning: A TMS study

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Experiments in rat primary motor cortex show that motor skill learning is associated with induction of synaptic long-term potentiation (LTP). The evidence was based on the observation that the efficacy of a protocol inducing LTP in the motor cortex was dramatically reduced after motor learning. Whether a similar principle applies in humans remains unknown. We used a recently established model of associative LTP and long-term depression (LTD) to examine the modifiability of motor cortical excitability following acquisition of a new motor skill. Subjects were asked to perform metronome-paced brisk isometric abductions with their right thumb at a defined force window adjusted to their individual maximum force in a series of 10 training sessions consisting of 50 contractions each. The increase of successful attempts was taken as a measure of motor learning. Paired associative stimulation (PAS) was performed by combining repetitively (0.1Hz, 90 pulses) electric stimulation of the right median nerve with subsequent tran-

scranial magnetic stimulation (TMS) over the left motor cortex at 25ms (PAS25) or 10ms (PAS10). LTP-like plasticity and LTD-like plasticity were induced by PAS25 and PAS10, respectively, and were evaluated before motor training by assessing motor cortex excitability changes. Single TMS pulses were used to elicit motor responses in the right abductor pollicis brevis (APB) muscle. The mean amplitude of 20 TMS-evoked potentials of APB was assessed before and after PAS on day 1 and day 2 in two different groups A (N=5) and B (N=6). At baseline (day 1 and day 2), LTP-like plasticity and LTD-like plasticity were similar in both groups (group A: PAS10: -20.3 ± 3.8% mean±s.e.m.; PAS25: 21.1 ± 6.9%, group B: PAS10: -16.0 ± 3.3%; PAS25: 24.5 ± 11.1%.). Similarly, neither training-induced motor performance improvement, nor increase of amplitudes of single-TMS evoked MEP (group A: 32.1 ± 19.2% ; group B: 39.0 ± 17.2%) differed significantly between both groups. Shortly after motor training on day 3, LTP-like plasticity could no longer be induced by PAS25 (% increase: -5.3 ± 11.2%; $p<0.05$) while LTD-like plasticity was similar as before training (% decrease -19.3 ± 9.7%; n.s.). These data suggest that motor cortical LTP-like plasticity had been driven to saturation by prior training. By contrast, the finding that LTD-like plasticity remained unchanged may reflect submaximal efficacy of the LTD-inducing PAS10-stimulation or involvement of additional cellular mechanisms. Together, these results are consistent with an important role of LTP in motor learning while leaving open the possibility of a contribution of alternative mechanisms.

Introductory Remarks to the Satellite Symposium D:

Novel properties of channels

Special Interest Group „Ionenkanäle" der Deutschen Physiologischen Gesellschaft

Klaus Benndorf, Heinrich Terlau and Frank Lehmann-Horn

Ion channels embedded in the plasma membrane of cells fulfil multiple physiological functions, including signal processing, secretion, or regulation of the cell volume. In the symposium recent data on novel channel proteins, channel activation mechanisms, and so far unknown channel functions will be presented.

Transient receptor potential proteins (TRP) form a family of Ca^{2+} permeable channels that are activated by a variety of signals as decreased intracellular Ca^{2+}, noxious thermal and chemical stimuli, and increased cell volume. Functional properties of TRPV4 channels, originally identified as osmotically activated channels, will be presented by C. Harteneck.

Cutaneous cold receptors are activated by the cooling of the skin and also by the application of menthol. Recently, one of the channels mediating the cold and menthol response has been identified and named CMR-1. This channel also belongs to the TRP-family (TRPM8). G. Reid will focus an the ionic channels involved in cold sensing.

Sensation of color by cone photoreceptors in the retina is mediated by cyclic nucleotide-gated (CNG) channels. Mutations in the A and B subunits of the CNG channels were identified to cause various forms of complete and incomplete color blindness (achromatopsia). Molecular mechanisms underlying these channelopathies will be presented by R. Seifert.

R. Blum will focus on the activation of TTX-insensitive $Na_v1.9$ sodium channels by neurotrophins, a surprising activation mechanism of sodium channels because these channels usually open in response to a voltage change across the membrane.

Pacemaker channels (hyperpolarization-activated pacemaker channels, HCN channels) have been cloned several years ago and it has become clear now that these channels are used by nature in different organs to induce rhythmical electrical activity. Properties and function of these channels in both the heart and the thalamus will be presented by M. Biel.

Characterisation of TRPV4 and potential functions 1223

Christian Harteneck

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Cation channels of the TRP family are calcium-permeable channels stimulated by hormonal and physical stimuli including hypoosmolarity. The functions of many isoforms, their activation mechanisms and physiological roles are unclear. In functional studies, heterologously expressed TRPV4 forms an osmotically activated cation channel, which has also been shown to be activated by 4 α-isomers of phorbol esters and is specifically blocked by ruthenium red. Expression analysis revealed strong signals in the kidney, where we demonstrated expression of TRPV4 in epithelial cells of the distal convolute. To study natively expressed TRPV4, we performed *in situ* hybridization to identify TRPV4 in cells already described as cell model; we localized TRPV4 in the plexus choroideus of mouse brain. The primary culture of porcine choroid plexus cells had been described in the literature and was adopted for functional studies. Imaging experiments revealed expression of a calcium entry mechanism with the pharmacological profile described for heterologously expressed TRPV4. For studies of TRPV4 in polarized epithelial cell layer with high transepithelial electrical resistance (TEER), the cells were cultured on Transwell supports, which allowed application of different media to either side of the epithelial cell layer. These experiments show the functional localization of TRPV4 at the apical side of polarized choroid plexus cells. On the background that the cerebrospinal fluid is produced by the choroid plexus cells by secretion, we propose a model in which TRPV4 senses the osmolarity of the secreted cerebrospinal fluid, and TRPV4-mediated calcium entry upon hypoosmotic activation produces an intracellular calcium signal necessary for feedback regulation to adjust cerebrospinal fluid to normal osmolarity.

Ion channels involved in cold sensing 1224

Gordon Gordon

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The detection of thermal stimlui in somatosensory neurones depends on members of the TRP (transient receptor potential) family of cation channels. The best known thermosensory molecule, the capsaicin receptor TRPV1 (VR1), was identified some years ago and along with its close relative TRPV2 (VRL1) can account in large part for the sensing of noxious heat. In the last year, the description of two more heat-sensitive channels (TRPV3 and TRPV4) and two cold-sensitive channels (TRPM8 and ANKTM1) has established a general role for TRP channels in thermal sensing. This talk will concentrate on the two cold-sensitive channels and on the native TRPM8-related current.

TRPM8 is a cold- and menthol-activated cation channel, which was found simultaneously by two quite different approaches: an expression-cloning based search for a menthol receptor, and a bioinformatics-based search for novel thermosensitive TRP channels. TRPM8 is distantly related to the heat-sensitive TRPV channels. Its properties are

essentially identical to those of the native cold- and menthol-activated channel which we had described in native dorsal root ganglion neurones.

We pre-selected cold-sensitive neurones by screening for a sudden, large increase in $[Ca^{2+}]_i$ at a distinct threshold temperature during cooling ramps. All of these rapidly responding cold-sensitive neurones expressed an inward current activated by cold and sensitised by (-)-menthol (and weakly by (+)-menthol). The current was absent from cold-insensitive neurones. Excised patches contained a cold- and menthol-activated channel, indicating that cold and menthol act directly to open the channel. As in intact cold receptors, lowered $[Ca^{2+}]_o$ sensitised the current, while raised $[Ca^{2+}]_o$ antagonised the menthol-induced sensitisation. During long cooling pulses the current showed adaptation, which depended on extracellular Ca^{2+} and was mediated by a rise in $[Ca^{2+}]_i$. This adaptation consisted of a shift in the temperature sensitivity of the channel. These properties - cold sensitivity, adaptation, and the effects of menthol and calcium - are similar to those of intact cold receptors, indicating that this current is the major mechanism of cold transduction in rapidly responding cold-sensitive DRG neurones.

However, a number of studies have reported a second class of cold-sensitive DRG neurone responding more slowly and at colder temperatures than TRPM8 or the native current described above, and insensitive to menthol. This suggested that there may be a second cold-sensing ion channel. Very recently a new cold-sensitive TRP-like channel has indeed been described, and called ANKTM1. It is a distant member of the TRP family, separate from the main TRPC, TRPV and TRPM subgroups, falling into a separate group with only one mammalian member. It shares the properties of responding to colder temperatures than TRPM8 and of being insensitive to menthol. Interestingly, it is co-expressed *in vivo* with a number of nociceptive markers - CGRP, IB4 binding, capsaicin sensitivity - which are not co-expressed with TRPM8 *in vivo*. This makes ANKTM1 a plausible candidate for a transducer of noxious cold stimuli, although this awaits confirmation from studies in native cold nociceptors.

1225 Cyclic Nucleotide-Gated Channels Involved in Color Blindness

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Cyclic nucleotide-gated (CNG) channels are nonselective ion channels that are opened by the direct binding of cyclic nucleotides, cAMP and cGMP. They form heterotetrameric complexes consisting of two or three different types of subunits. Six different genes encoding CNG channels, four A subunits (A1 to A4) and two B subunits (B1 and B3) have been identified. The CNG channel of cones consists of A3 and B3 subunits, probably in a 3:1 stoichiometry. Genetic analysis of patients suffering from autosomal recessively inherited achromatopsia revealed, that the disease can be caused by mutations in the genes coding for the A3 and B3 subunits. In two siblings suffering from incomplete achromatopsia, both A3 alleles are mutated. One mutation leads to a substitution of Thr to Arg in the S2-S3-linker, the other mutation leads to a substitution of Thr to Ser in the P-region of A3. Heterologous expression of the subunits in HEK293 cells revealed that only the P-region mutant forms functional channels. Compared to the

wildtype channel, the P-region mutant displayed 1) increased single-channel conductance, 2) reduced apparent affinity of the pore for extracellular Ca^{2+}, 3) altered gating and 4) substantially reduced ligand sensitivity. Quite surprisingly co-expression of the mutant A3 subunit with the B3 subunit completely rescues the gating phenotype and restores the native ligand sensitivity and the conductance of monovalent ions. However the apparent affinity for external Ca^{2+} is even further impaired in heteromeric channels incorporating the mutant A3 subunit. Our results suggest that a change in Ca^{2+} homeostasis causes alterations in color vision in the affected siblings.

$Na_v1.9$, a sodium channel involved in neurotrophin-evoked depolarization

1226

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Neurotrophins, including brain-derived neurotrophic factor (BDNF), are essential for the normal functioning of the mammalian nervous system. A rapid, transmitter-like neuroexcitatory action of BDNF is found in many types of central neurons (Kafitz et al. *Nature* 401, 1999) and has been recently implicated in synaptic plasticity (Kovalchuk et al. *Science* 295, 2002). Here we demonstrate the molecular reconstitution of the BDNF-mediated current (I_{BDNF}). By screening candidate transcripts with antisense mRNA expression in SH-SY5Y neuroblastoma cells, we identified the tetrodotoxin (TTX)-insensitive sodium channel $Na_v1.9$ (NaN, Scn12a) and the receptor tyrosine kinase TrkB as necessary molecular constituents for I_{BDNF}. In HEK293 cells, stimulation of recombinant TrkB receptors by BDNF activated the co-expressed sodium channel $Na_v1.9$. As found for I_{BDNF} in hippocampal neurons and in SH-SY5Y cells, the reconstituted current was insensitive to TTX, however highly sensitive to the sodium channel blocker saxitoxin. The homologous voltage-gated sodium channel $Na_v1.7$ was not able to replace the function of $Na_v1.9$ in evoking I_{BDNF}. By analyzing the voltage-dependence of I_{BDNF} in hippocampal and in HEK293 cells, we found that I_{BDNF} reversed at the calculated reversal potential for sodium ions. Moreover, in both cell types the inward current gradually decreased at membrane potentials below -65 mV and disappeared at -110 mV. Quantitative real time RT-PCR verified the abundant expression of $Na_v1.9$ (NaN) in different brain areas. However, in tissue from rat hippocampus, cerebellum and cortex, the expression level was shown to be about 100-fold lower as in dorsal root ganglia, where $Na_v1.9$ was thought to be primarily expressed. Consequently, the targeted elimination of $Na_v1.9$ by antisense mRNA expression in cultured hippocampal neurons also blocked BDNF-evoked depolarization. Recent experiments also indicate that $Na_v1.9$ can act as a modulator of BDNF-induced CREB phosphorylation. This suggests a role for this sodium channel in the regulation of BDNF-induced, activity-dependent gene expression. All together, our results demonstrate that $Na_v1.9$ is a unique type of sodium channel that is gated by the extracellular ligands BDNF or NT-4/5, the most powerful neuroexcitants reported so far (Blum et al. *Nature* 419, 2002).

1227 Pacemaker channels of heart and thalamus

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The hyperpolarization-activated cation current (I_h) is believed to play a key role in the generation of cardiac pacemaker depolarizations as well as in the control of neuronal excitability and plasticity. The channels underlying I_h, termed hyperpolarization-activated cyclic nucleotide-gated channels (HCN1-4), have been recently identified by molecular cloning. HCN channels are members of the superfamily of voltage-gated cation channels. Like with other members of this family, it is very likely that four HCN channel subunits assemble to a tetrameric channel complex. All four HCN channel subunits are expressed in brain whereas in heart cells only HCN2 and HCN4 are present in significant amounts. *In situ* hybridization and immunocytochemistry indicate that the expression pattern of HCN1-4 in brain are quite diverse and only partly overlapping. While this finding may suggest that HCN channels exist as homotetramers there is also experimental evidence that HCN channel subunits can form heteromeric complexes *in vivo*. In heterologous expression systems all four HCN channel subunits induce currents with the principal properties of I_h. So far, it is not known which particular role each individual HCN channel isoform fulfils in various types of neurons and heart cells. To address this issue we have deleted the HCN2 channel in the mouse genome by gene targeting. HCN2-deficient mice exhibit spontaneous absence seizures. The thalamocortical relay neurons of these mice display a near complete loss of the HCN current, resulting in a pronounced hyperpolarizing shift of the resting membrane potential, an altered response to depolarizing inputs and an increased susceptibility for oscillations. HCN2-null mice also display cardiac sinus arrhythmia, a reduction of the sinoatrial HCN current and a shift of the maximum diastolic potential to hyperpolarized values. Mice with cardiomyocyte-specific deletion of HCN2 display the same arrhythmia as mice lacking HCN2 globally, indicating that the arrhythmia is indeed caused by sinoatrial dysfunction. Our results define the physiological role of the HCN2 subunit as a major determinant of membrane resting potential that is required for regular cardiac and neuronal rhythmicity.

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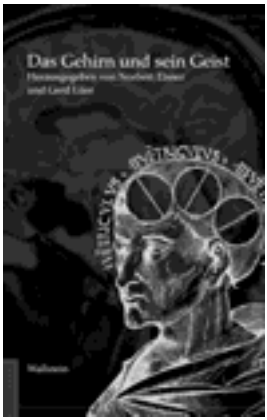

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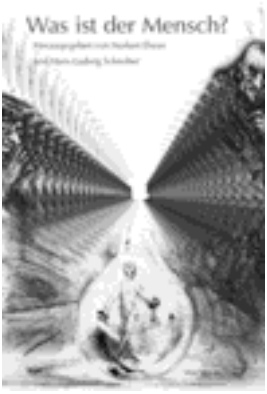

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